AN ASSESSMENT OF OCCUPATIONAL EXPOSURE TO GRAM-NEGATIVE ORGANISMS IN AN URBAN POULTRY SLAUGHTER AND PROCESSING PLANT IN COLUMBIA, SC, USA

by
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Abstract

Background: Poultry slaughter / processing plants process large numbers of birds from multiple industrial feeding operations, where the birds are raised in crowded, confined conditions and commonly fed sub-therapeutic doses of antimicrobials. As plant workers are in close contact with large numbers of live birds and fresh carcasses, these environments are conducive to the transfer of bacteria, including antimicrobial resistant bacteria, from the animals to the workers. Due to the increasing frequency of antimicrobial resistant bacteria, better understanding of the sources of human exposure to these organisms is needed.

Objectives: Using job duties as a surrogate for contact with broilers, this analysis assesses the association between occupational contact with broilers and nasal carriage of gram-negative organisms (GNOs) in workers at a chicken slaughter and processing plant. The occupational contact with broilers could be direct or via bioaerosols. Antimicrobial susceptibility patterns of the isolates from the most frequently detected genera are also qualitatively explored.

Methods: The data analyzed is a subset of data from a cross-sectional exploratory study of poultry slaughter / processing plant workers in Columbia, South Carolina. Questionnaire data and nasal swabs were collected from participants. Nasal swabs were tested for *S.aureus* and GNOs; isolates were screened for antimicrobial susceptibility. For the analysis, participants were categorized based on job duties, as reported through the questionnaire. The association between job categories and nasal GNO carriage was analyzed using logistic regression models.

Results: Out of the 90 participants analyzed, thirty-six (40%) were positive for nasal GNOs. Nearly a third (9/29) of the tested isolates displayed antimicrobial non-susceptibility. Compared to participants with intermittent or infrequent poultry contact – namely those with office, shipping, or packing duties – the adjusted odds ratio of GNO carriage was 6.29 times (95% CI: 1.43, 27.71) and 5.90 times (95% CI: 0.94, 37.50) higher, respectively, in participants with the most frequent poultry contact and workers conducting maintenance/cleaning.

Conclusions: These findings suggest that poultry slaughter / processing plant workers in frequent contact with live poultry and/or carcasses and those conducting cleanup and maintenance are likely at increased risk of exposure to GNOs, including antibiotic resistant GNOs, as compared to workers with less frequent or no poultry contact.

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isolates from each genus while box height reflects the percentage of isolates from each job category. Of these participants, 34 were positive for GNOs but not *S. aureus*, 2 were positive for both GNOs and *S. aureus*, and 12 were positive for *S. aureus* but not GNOs.

Figure 3. Antimicrobial susceptibility pattern of the tested gram-negative organism (GNO) isolates. Forty GNO isolates were isolated from the nares of 36 out of the 90 participants, one per individual for 32 participants and 2 morphologically distinct isolates per individual for 4 participants. Of these isolates, we tested the antimicrobial susceptibility of isolates from the most frequently detected genera (*Acinetobacter* (n=10, 1 additional did not grow), *Citrobacter* (n=7), *Enterobacter* (n=4), *Proteus* (n=4), *Pseudomonas* (n=4, 1 additional did not grow). As indicated in the methods, the antimicrobials used in testing were based on the CLSI standard and consultation with the Johns Hopkins Hospital Clinical Microbiology Laboratory. Twenty out of the tested 29 isolates were pan-susceptible to all of the tested antimicrobials; 9 of the 29 isolates (31.0%) were non-susceptible to at least one antimicrobial. All *Enterobacter* and *Citrobacter* isolates were pan-susceptible to all of the tested antimicrobials and are not included in the figure below; the antimicrobial susceptibility patterns of the remaining 18 isolates are depicted in the figure. Some drugs were not tested for all genera due to intrinsic resistance or to not being recommended for those genera.

Figure 4. Graphical display of the odds /adjusted odds ratios and 95% confidence intervals for nasal GNO carriage, by the job categories, in univariate, final adjusted, and alternative adjusted models. Purple corresponds to the estimates for the pre-slaughter / processing job category; blue corresponds to the estimates for the maintenance / cleaning category. The shaded areas show ±10% of the univariate odds ratio; horizontal lines at the null value (red) and the univariate values (dashed lines, color coded) are included in the graph for reference. A red arrow is added to the graph to show the final model.

Abbreviations: Recruit, recruitment round; AB, self-reported antibiotic usage in the last 6 months; shift, 3rd shift (shift after plant cleaning) or earlier shift.

Introduction

General Background: Public Health Significance of Broiler Production

Implications of Broiler Production Methods on Human Infectious Diseases in the US

In the United States, as in many other developed countries, methods of raising and slaughtering food-producing animals have been dramatically transformed since the first half of the twentieth century (MacDonald & McBride, 2009; Pew Commission, 2008). These unprecedented transformations include more specialized, intensified production and increasing flock / herd sizes. This includes the proliferation of large Animal Feeding Operations (AFOs) and Concentrated Animal Feeding Operations (CAFOs), which are defined by regulations as facilities where animals of any species are provided feed (as opposed to foraging on vegetation) and confined in the same buildings as their waste for at least 45 days of the year (Environmental Protection Agency, 2014). Broilers, which are chickens bred and raised for meat, raised in AFOs or CAFOs are typically confined in these facilities for the entirety of their lifespan. Most food-producing animals in the US are now fed in confined conditions (MacDonald & McBride, 2009) and sub-therapeutic doses of antimicrobials are commonly administered to the animals, mostly by addition to feeds, in order to prevent disease and promote growth. This is especially true for swine and for broilers, which are chickens bred and raised for meat (Davis, Price, Liu, & Silbergeld, 2011; MacDonald & McBride, 2009). Generally speaking, large food-producing animal operations tend to use antimicrobials more intensively than smaller ones (MacDonald & McBride, 2009).

Within the food-producing animal industry, broiler production is a large, highly industrialized subset. In 2014, a United States Department of Agriculture (USDA) report noted that per-capita poultry consumption in the US has grew rapidly from the 1960s to 2003, with slower growth since 2003 and slight declines in 2009 and 2012. Per-capita chicken consumption, of which nearly all are broilers, exceeds beef and pork consumption (MacDonald, 2014). The most recently available USDA census (USDA, 2014), notes over 8.4 billion broilers and other

meat-type chickens were sold in 2012. This corresponds to a 2.4 fold increase from the numbers sold twenty years ago (3.5 billion in 1982). Transformation of the broiler industry began earlier than comparable industries of other food-producing animals, with the current structure and processes being established in the 1950s and 1960s. Because the transformation of the broiler industry was well underway in the 1960s, increases in the size of the broiler production locus (defined as the median of farm size distribution, weighted by production) within the last 20-30 years, while still remarkable, are smaller than changes from the same time period in some of the other food-producing animal industries and also smaller than the earlier changes within the broiler industry. Nevertheless, the broiler industry continues to trend towards larger facilities managed by farms producing increasingly larger numbers of animals. In 2011, the average broiler came from a farm that sold 628,600 broilers (MacDonald, 2014); an equivalent broiler in 1987 came from a farm that sold 300,000 broilers (MacDonald & McBride, 2009). Broiler production is highly concentrated in the Southeastern and Mid-Atlantic regions of the US (Leibler, Carone, & Silbergeld, 2010).

As in the industrial production of several other food-producing species, it is common for the operational groups involved in broiler production to further specialize to single stages of the process. Because the production stages are located in different sites, repeated transportation of the animals is needed (Leibler et al., 2009). The process of producing and delivering broiler products to consumers can be broken down into six stages: breeder farms, hatchery farms, grow-out farms, slaughter plant, further processing (for some types of products), and retail / food service / exports. The integrator firms, which typically own the hatcheries, processing plants, and feed mills, contract with separate grow-out farms (MacDonald, 2014; Ollinger, MacDonald, & Madison, 2005). The broiler industry is highly integrated; in 2011, over 97% of broilers were raised on contract operations (MacDonald, 2014). Under contract, the integrators provide chicks to the grow-out farms; the chicks are raised on the grow-out farm for five to nine weeks before being sent to slaughter, depending on the desired size of the bird (MacDonald, 2014). Grow-out farms

are typically within a few hours' driving distance from the slaughter / processing plant; per the 2011 USDA census, 90% of broilers were grown on farms within 60 miles of the plant (MacDonald, 2014).

In addition to the numerous environmental and sustainability issues related to the methods of industrial food animal production (Pew Commission, 2008; Tilman, Cassman, Matson, Naylor, & Polasky, 2002), these methods raise concerns of transmission of viral and bacterial pathogens and the spread of antibiotic resistance. From the perspective of emerging infectious diseases, industrial food animal production systems can be considered as a distinct, anthropogenic ecosystem (Davis et al., 2011; Leibler et al., 2009). Concentrating relatively large numbers of animals in small, waste-filled spaces creates environments conducive to the proliferation, and possible evolution of, bacteria and viruses that colonize or infect these animals (Leibler et al., 2009; Liverani et al., 2013). Earlier in the transformation of industrial food animal production, when these intensified methods started to be implemented, transmission of infectious diseases amongst the densely-confined animals caused problems for the agricultural industry. This partially contributed to the introduction of feeding sub-therapeutic prophylactic antimicrobials to the animals (Karesh et al., 2012); a practice that has been thought to both promote growth and prevent disease (MacDonald, 2014). Consistent with this, poultry confinement buildings are known to have high concentrations of a wide variety of airborne bacteria and fungi (Lawniczek-Walczyk, Gorny, Golofit-Szymczak, Niesler, & Wlazlo, 2013; Nonnenmann, Bextine, Dowd, Gilmore, & Levin, 2010). The density of airborne microorganisms in poultry confinement buildings has been shown to vary with broiler age (Lawniczek-Walczyk et al., 2013; Oppliger, Charriere, Droz, & Rinsoz, 2008), season of year (Lawniczek-Walczyk et al., 2013), and other environmental factors (Lawniczek-Walczyk et al., 2013; Nonnenmann et al., 2010). Similar to other food-producing animals, the genetic diversity of US broilers is limited (Davis et al., 2011), which may favor increased adaptation and transmission of pathogens (Davis et al., 2011; Jones et al., 2013; Liverani et al., 2013). As described in the subsequent paragraphs,

these factory-like conditions increase the likelihood of human exposures to zoonotic bacterial and viral pathogens in several ways.

Zoonotic diseases are highly relevant to public health. As reviewed by Karesh et al (2012), it has been estimated that over 60% of infectious diseases in humans are caused by pathogens shared with wild or domesticated animals; furthermore, most of the emerging infectious diseases that have been identified in the past 70 years are zoonotic. Past large-scale changes impacting biodiversity and animal-human interactions have been linked to many zoonoses. Not surprisingly, some of the bacteria and viruses that proliferate in these high animal density settings can be pathogenic to humans. For example, many pathogens causing foodborne diseases are enzootic in livestock (Karesh et al., 2012). Thus, in addition to a heightened risk of disease spread within the food-producing animals themselves, the conditions in industrial food animal production result in a higher risk for pathogen transmission to humans (Gilchrist et al., 2007; Jones et al., 2013; Sarmah, Meyer, & Boxall, 2006; Silbergeld, Graham, & Price, 2008). Furthermore, food-producing animals can serve as intermediate or amplifying hosts for disease from wildlife species, so this risk is not limited to pathogens for which food-producing animals are the main reservoir (Jones et al., 2013; Liverani et al., 2013). Workers in poultry growing facilities are regularly exposed to concentrations of airborne microorganisms in excess of 10^6 colony forming units (CFU) per cubic meter (Lawniczek-Walczyk et al., 2013). The dusts and bioaerosols contributing to these high concentrations of airborne microorganisms are thought to have a negative impact on workers' respiratory health (Donham, Cumro, & Reynolds, 2002; Lawniczek-Walczyk et al., 2013; Nonnenmann et al., 2010; Oppliger et al., 2008). In addition, the Center for Disease Control and Prevention's National Institute for Occupational Safety and Health (CDC NIOSH) notes workers in poultry production are at increased risk of avian influenza, *Campylobacter jejuni, Chlamydia psittaci, Escherichia coli*, and *Salmonella* infections(Centers for Disease Control, 2014).

There is evidence that more antibiotics are used in food animal production than in humans (CDC, 2013a). The most recently available Food and Drug Administration (FDA) report on approved antimicrobials for food-producing animals, which covers sales and distribution in 2013, notes that approximately 14.9 million kilograms of antimicrobials approved for use in food-producing animals were sold and distributed domestically. Of these antimicrobials, 62% meet the FDA definition of medically important for humans; the remaining 38% belong to the drug classes of aminocoumarins, pleuromutilins, polypeptides, quinoxalines, glycolipids, and ionophores. Approximately 74% of these 'medically important antimicrobials' were sold / distributed for feed administration (this corresponds to 46% of all the antimicrobials) (Center For Veterinary Medicine, FDA, 2015), which results in highly imprecise dosing of the animal and therefore may further facilitate selection for antimicrobial resistance (Love, Davis, Bassett, Gunther, & Nachman, 2011). Most of the rest these 'medically important antimicrobials' (approximately 21%) were sold / distributed for administration by water (this corresponds to 13% of all the antimicrobials). Only 5% were sold / distributed for administration by other routes. Reports on the sales and distribution by animal species or on the amounts actually administered or used are not available (Center For Veterinary Medicine, FDA, 2015), which precludes more granular estimates of the extent of antimicrobial usage in food-producing animals. For poultry, the antimicrobials approved for oral administration include many of the antimicrobials in World Health Organization's categories of critically important clinical antimicrobials (aminoglycosides; macrolides; ketolides), highly important clinical antimicrobials (lincosamides; streptogramins; sulfonamides, dihydrofolate reductase inhibitors and combinations; tetracyclines), and important clinical antimicrobials (aminocyclitols and cyclic polypeptides) (Durso & Cook, 2014).

For bacterial pathogens, widespread usage of antimicrobials in industrial food animal production selects for the development of antimicrobial resistance. Many bacterial antimicrobial resistance genes are on mobile elements, and conjugation is thought to be the main mechanism by which bacteria transfer antimicrobial resistance genes (Silbergeld, Graham et al., 2008; Verraes et

al., 2013). As mobile genetic elements can be exchanged across a wide array of bacterial species, and can provide resistance to multiple types of antimicrobials, this widespread selection for antimicrobial resistance increases the size of environmental reservoirs of antimicrobial resistance, which in turn increases the potential for further propagation of antibiotic resistance (Silbergeld, Graham et al., 2008). An ever-growing body of literature characterizes the implications of widespread feed-administered non-therapeutic antimicrobial use in food-animal production (Anderson, Nelson, Rossiter, & Angulo, 2003; Davis et al., 2011; Doyle, Loneragan, Scott, & Singer, 2013; Durso & Cook, 2014; Gilchrist et al., 2007; Jones et al., 2013; Karesh et al., 2012; Marshall & Levy, 2011; Sarmah et al., 2006; Silbergeld, Davis, Leibler, & Peterson, 2008; Silbergeld, Graham et al., 2008; Singer & Williams-Nguyen, 2014). As reviewed in the these articles, numerous studies have linked antibiotic resistant strains to industrial animal food production facilities, found differences in the prevalence of antibiotic resistance between food-producing animal facilities that administer antibiotics extensively to their animals compared to facilities that do not, and documented human exposure to antibiotic resistant strains matching the strains found on livestock. Despite these observations, which provide strong cause for concern, much remains to be understood about the transmission dynamics and ecology of agriculture-associated antimicrobial resistance. The growing body of evidence and concern over antimicrobial misuse in food-producing animals has led to the recent release of regulatory agency guidelines to begin to curb their usage in these settings (Food and Drug Administration, 2014).

The complex transportation chains that characterize industrial food production create additional pathways for direct pathogen transmission to a wider range of individuals than those who work in the growing facilities (Jones et al., 2013; Leibler et al., 2009; Liverani et al., 2013), such as individuals working in transportation or the slaughter / processing of the animals, veterinarians and other animal health workers (R. C. Neyra, Vegosen, Davis, Price, & Silbergeld, 2012). This risk, in turn, extends to their families and communities (R. C. Neyra et al., 2012). Beyond this, ventilation systems, waste removal, water contamination, animal-to-animal, and

animal-to-insect contact provide additional indirect or environmental pathways for pathogen transmission to humans who are not directly involved in the production / transportation chain (Jones et al., 2013; Liverani et al., 2013; Silbergeld et al., 2008). Livestock density has been shown to be a risk factor for community-level exposure to pathogens associated with food-producing animals (Feingold et al., 2012). A recent study of one livestock associated pathogen, *S.aureus* CC398, suggests it has moved into hospital settings and, in those settings, can be transmitted from human to human (Ward et al., 2014). Environmental spread of antimicrobial resistant bacteria, as well as spread of the antibiotic residues into the environment (estimates indicate that 30-90% of antibiotics administered to animals are excreted (Karesh et al., 2012)), can potentially contribute to the spread of antibiotic resistance in the poorly understood environmental reservoirs of antibiotic resistance (Allen et al., 2010; Davis et al., 2011).

Surveillance and Monitoring Related to Broiler Production in the US

In the US, there are numerous mechanisms in place, which are intended to assure and monitor the safety of retail meat, the end product of industrial food animal production. These include USDA inspections and HACCP requirements. Additionally, through a number of programs under the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS), national surveillance and monitoring is done among food-producing animals for a subset of diseases (Animal and Plant Health Inspection Service, United States Department of Agriculture, 2015). For poultry, the USDA conducts active surveillance for avian influenza and enforces avian influenza testing in live bird markets, live bird distributors, and live bird production facilities (USDA APHIS, 2012). However, despite the risks noted above to workers and communities involved in industrial food animal production, there currently is not systematic monitoring and surveillance of pathogen exposures or outbreaks in these populations. Within CDC NIOSH, the Office of Agricultural Safety and Health (OASH) is involved in a number of projects characterizing exposure to avian influenza, livestock associated MRSA, and endotoxins / dust in settings where food-producing animals are raised and harvested, but

surveillance related to pathogen exposure in these settings does not occur (Office of Agricultural Safety and Health, Centers for Disease Control, 2012). Additionally, CDC NIOSH has a separate office for poultry industry workers, which provides health-related resources for employers and workers within the poultry industry and does targeted assessments and reporting, but does not conduct systematic surveillance (Centers for Disease Control, 2014).

In the US, antimicrobial resistance surveillance is also limited in settings of food-producing animals. Systematic data collection of antibiotic usage within food-producing animals is not collected (CDC, 2013a). As noted above, while the FDA receives annual reports on the distribution and sales of antimicrobials for usage in food animals, this data has strong limitations and, although summarized by the FDA, the data itself is not made publicly available (Center For Veterinary Medicine, FDA, 2015). In its 2004 hog and 2006 broiler versions of the Agricultural Resource Management Survey, the USDA included questions about antibiotic usage; however, the intent of this data collection was only to identify the extent of sub-therapeutic antibiotic usage, its impacts on productivity, and assess potential alternatives, not to assess resistance or health hazards (MacDonald & McBride, 2009). Aside from the networks dedicated to monitoring resistance in gonorrhea and tuberculosis, the networks focused on non-nosocomial antimicrobial resistance are the Active Bacterial Core, FoodNet, and National Antimicrobial Resistance Monitoring System (NARMS) (Centers for Disease Control, 2013). The scope of FoodNet is limited to a subset of foodborne illnesses (CDC, 2013b); as such, it can only be expected to detect trends only in those pathogens that are transmitted through food, and not the ones transmitted via other mechanisms. Although NARMS monitors trends of antimicrobial resistance by sampling from retail meats, food-producing animals, and humans, its monitoring in retail food and food-producing animals is limited to *Salmonella*, *Campylobacter*, *E.coli*, and *Enterococcus.* Its monitoring in humans also includes *Vibrio* species and *Shigella* (Food and Drug Administration, 2013). The human arm only detects cases that present to clinical care; an external subcommittee of the FDA's Scientific Advisory Board has recommend the surveillance be extended to sample

from healthy individuals as well; however, to date, this has not materialized (Doyle et al., 2013). Active Bacterial Core does surveillance for invasive disease caused by select bacteria (CDC, 2014); similarly to NARMS, cases must present to a healthcare facility to be detected. There is no targeted sampling or surveillance of workers or communities in the food-producing animal industry.

Study Introduction

The US Chicken Slaughter / Processing Plant Environment

Like other components of the US food animal industry, the chicken slaughter and processing industry has been transformed in several ways since the 1950s. Dramatically increasing consumer demand for chicken meat, and shifts in consumer preferences from whole fryers to more processed and convenient forms of chicken meat (for example, deboned meat or nuggets) has been accompanied by notable increases in plant size (Ollinger, MacDonald, & Madison, 2000), with the mean plant size nearly tripling between 1967 and 1992 (Ollinger et al., 2005). Based on 2011 and 2006 data, plants slaughter about 1.1 million broilers per week, on average (MacDonald, 2014). Broilers are supplied from multiple growing facilities (Ollinger et al., 2000; Ollinger et al., 2005) and many of the plants run 19-20 hours a day with the remaining hours set aside for cleaning / disinfection activities (Corry & Atabay, 2001).

CDC NIOSH and the Occupational Safety and Health Administration (OSHA) provide descriptions of typical workflows and job duties, respectively, in poultry processing plants (Centers for Disease Control, 2014; OSHA,). Briefly, live poultry are unloaded from open crates from the transport trucks, then manually hung by their feet to a shackle conveyor. Stunning, killing, and de-feathering of the birds occurs next, typically though automated processes with employees doing back-up killings of any birds missed by the machine. The feet or 'paws' are then severed, thus removing the carcass from the kill conveyor line. Paws are diverted to a separate conveyor for sorting and the carcasses are rehung to the evisceration line. During the evisceration stage, carcasses are cut open; the necks are severed; internal organs are removed in a piecemeal, specialized fashion (some of these organs are further reserved for USDA inspection); and the subset of usable internal organs (e.g. heart, liver, gizzards) are diverted for washing, inspection, and packaging. The carcasses are then sent through chiller baths with antimicrobial agents to reduce bacteria load. Next, carcasses are diverted either to packaging (for whole bird products), cutting (for bone-in products), or deboning (bone-out products). Cutting is typically done using a

'cone line', which is an assembly line containing cone-shaped stages on which the individual carcasses are mounted. Depending on the part of meat, deboning occurs on the cone line at the end of the cutting process (e.g. for the breast) or on a separate line. Packaging of the product usually occurs in two steps: the product is put into its packaging, then the packaged product is placed into its shipping box. Job descriptions related to packaging include: assembling the boxes, putting poultry products into the boxes, adjusting the weight within the boxes (includes manual addition or removal of product until proper weight is attained), sealing the boxes, and removing the sealed boxes. The sealed boxes are then loaded to trucks or shelves for storage. OSHA notes two types of sanitation workers: those who work during the production shifts, who are frequently entry-level workers tasked with keeping the machinery and floor clean during production; and those who do the daily clean-up outside of the production shifts to comply with USDA food safety inspection requirements for the plant contact surfaces. In some regions of the country, the daily clean-up crew tend to be plant employees, while in other regions of the country, they tend to be contractors.

From the perspective of infectious diseases, chicken slaughter / processing plants represents a unique environment. Broiler flocks have diverse, large microbial communities (Kotula & Pandya, 1995). At the plant, the communities of numerous, large, dirty, and separately reared flocks are brought together. The transportation process to the plant is stressful for the flocks and has been associated with increased shedding of pathogens like *C.psittaci* (Deschuyffeleer et al., 2012) and *Campylobacter* spp. (P. Whyte, Collins, McGill, Monahan, & O'Mahony, 2001b). As organism shedding patterns can change following certain stressors that occur during transportation (Mulder, 1995), it is therefore possible that transportation to the slaughtering facility results in increased shedding of any pathogens or antimicrobial resistant bacteria that are part of the broilers' microbial communities. The microbial communities of different sites of the broiler differ – for example, the extensive microbiota of the feathers, skin, and feet (Cason et al., 2007) differs from the extensive microbiota of the gastrointestinal tract

(Rehman, Vahjen, Awad, & Zentek, 2007; Torok, Allison, Percy, Ophel-Keller, & Hughes, 2011). While the broilers are slaughtered and processed, the internal and external body parts of the birds, and also the microbial communities associated with these body parts, become exposed to each other, which provides further opportunity for microbial mixing.

As briefly noted above, numerous required control measures, which are overseen by the USDA, are in place to reduce microbial load on the carcasses as they progress through processing so that the food products can be safely sold (United States Department of Agriculture, 2014). Despite these measures, the targeted pathogens and microbes are not completely controlled; thus they persist in the chicken slaughter / processing plant environment and in the chicken products themselves (Government Accountability Office, 2014). Studies of foodborne pathogens have demonstrated that if a 'pathogen-positive' flock is processed ahead of a 'pathogen-negative' flock, cross-contamination of carcasses or meat from 'pathogen-negative' flocks with the pathogens detected from the 'pathogen-positive' flock occurs (Corry & Atabay, 2001; Genigeorgis, Hassuneh, & Collins, 1986; Rasschaert, Houf, & De Zutter, 2007). The detected species and cell count patterns of meat spoilage bacteria on the carcasses at various stages of the processing cycle reveal cross-contamination throughout processing, even though the detected bacterial load of the processed carcasses is significantly less than the detected bacterial load in carcasses entering the processing line (Hinton, Cason, & Ingram, 2004). In a study of MRSA in a Dutch chicken slaughterhouse, increases of plant MRSA contamination over the course of the working day were seen (Mulders et al., 2010). Other studies have noted the plant itself as a source of product contamination, separately from the birds being currently processed that day. This has been noted for spoilage lactic acid bacteria (Vihavainen et al., 2007) and *C.jejuni* (Johnsen, Kruse, & Hofshagen, 2006). Although ideal conditions for proliferation differ by bacterial species, the continued presence of targeted microbes on the poultry carcasses and chicken meat throughout the slaughter / processing plant process implies that other, not measured, bacterial species, potentially including other zoonotic pathogens, also persist.

Evidence of the extensive microbial presence within the chicken slaughter / processing plant environment is also provided by air sampling studies; many of which are done to identify mechanisms to prevent cross-contamination of the poultry products as they progress throughout the processing line. A study in a chicken slaughterhouse in China found mean airborne bacterial levels of $1.5x10^5$ CFU/m^3 in the receiving-hanging area of the plant. Airborne bacterial levels were lower later in the assembly line, and for the types of bacteria analyzed (aerobic bacteria, *S.aureus,* total coliforms, *E.coli*, *P.aeruginosa, L.monocytogenes, B.cereus,* and *Salmonella*), the predominant bacteria varied in different parts of the plant (Liang et al., 2013). A study conducted by a German investigator found mean counts of airborne *Enterobacteriaceae* up to $\log_{10}$ 3.24 CFU/m^3 in the chicken reception area, with lower counts in other parts of the plant, and detected different predominant species in the airborne microflora at different parts of the production line (Ellerbroek, 1997). In a South Korean study, the highest detected levels of total airborne microorganisms were observed in the receiving-killing areas and defeathering areas. The species analyzed, and their highest detected airborne levels, were: *S.aureus* (10^4 cfu/m^3), total coliforms ($1.6x10^3$ cfu/m^3), *Salmonella* species (5.5×10^3 cfu/m^3), *P.aeruginosa* (1.9×10^3 cfu/m^3), *L.monocytogenes* (9.3×10^3 cfu/m^3), *B.cereus* (1.8×10^4 cfu/m^3) (Lues, Theron, Venter, & Rasephei, 2007). Another study detected mean counts of airborne *Enterobacteriaceae* as high as $\log_{10}$ 1.63 CFU/$foot^3$, with lower levels in other parts of the plant (P. Whyte, Collins, McGill, Monahan, & O'Mahony, 2001a).

From the perspective of antimicrobial resistance, the impact of the stressors that bacteria in the slaughter / processing plant environments face, due to the cleaning / decontamination procedures, and the presence of biofilms on processing equipment warrant consideration as well. Certain bacterial stressors, such as stress caused by heat or cold, may trigger changes in phenotypic antimicrobial resistance and increase transfers of genetic material between bacteria (Verraes et al., 2013). Bacteria surviving cleaning / decontamination steps within the slaughter / processing plant are likely to enter this stressed state. DNA, including any resistance genes,

released from lysed, killed bacteria could also theoretically be acquired by nearby bacteria via transformation. Biofilms, which provide ideal conditions for exchange of genetic material among the bacteria within the biofilm and are associated with increased levels of antimicrobial resistance, are known to form on processing equipment within the food industry (Verraes et al., 2013). Several factors within poultry processing plants, such as the complex, difficult to clean machinery; large quantities of carcasses being processed; and long production cycles favor the formation of biofilms. Furthermore, it is recognized that standard cleaning / disinfection procedures might not be adequate for their removal, and that bacteria can detach from biofilms via contact or aerosols (Giaouris et al., 2014).

Approximately 220,000 individuals are employed in poultry processing (includes chicken and other types of poultry) in the United States (BLS, 2013). Work conditions in poultry processing plants involve many repetitive movements, physically demanding activities, cold temperatures, quickly moving production lines, dangerous machinery, and numerous sharp tools. These conditions are recognized as extremely hazardous; safety training is complicated by high rates of employee turnover (Government Accountability Office, 2005). In addition to the recognized substantial musculoskeletal, chemical, and injuries hazards, hazards from certain microbe and pathogen exposures, notably *Camplyobacter jejuni, Chlamydophila psittaci,* (more common in plants that process other types of poultry)*, E.coli*, Salmonella, and endotoxins are also recognized as substantial by US government agencies (Centers for Disease Control, 2014). Currently in-place national monitoring and inspection laws target the food products themselves and the worker injuries, illnesses, and severe incidents (like fatalities) that are reported by employers through OSHA forms. Although OSHA does not have additional unique industry standards for the poultry processing industry (OSHA,), mechanisms are in place for USDA product inspectors to notify OSHA of potential hazards to the workers; these inspectors also receive training on awareness of zoonotic diseases (Government Accountability Office, 2005).

Employer reporting of worker injuries and illnesses to OSHA are widely believed to be underreported (Government Accountability Office, 2005); surveys of occupational injuries and illnesses done by the Bureau of Labor Statistics (BLS) are also believed to be underreports (Boden & Ozonoff, 2008). In the context of poultry slaughter / processing plant environments, underreporting may be worsened due to incentives in place at the plant and the undocumented status of some of the workforce. Additionally, as cleaning and sanitation workers are not classified by BLS as working in the industry, reported illnesses and injuries from these workers are not counted in estimates for the poultry slaughter / processing plant industry (Government Accountability Office, 2005). Injuries not requiring more treatment than first aid do not need to be reported (Kyerematenng-Amoah, Nowell, Lutty, Lees, & Silbergeld, 2014); therefore it is reasonable to expect that minor work-related cuts or lacerations, or pre-existing cuts or lacerations, which become infected due to pathogen exposure on the job would not be captured by this system. Furthermore, determining whether an infectious disease was contracted at work is difficult, and the criteria for determining whether an illness is work-related (C.F.R., 2001) likely would not capture mild to moderate illnesses resulting from exposure to pathogens at the plant and certainly would not capture asymptomatic colonization or carriage of a pathogen contracted at the plant.

Pathogen exposures in this setting is particularly concerning in light of the high injury and laceration rates among chicken slaughter / processing plant workers. For certain pathogens, notably *S.aureus* which causes skin infections, the combination of the high injury / laceration rate along with pathogen exposures would likely put the workers at increased risk of developing infected wounds or other opportunistic infections (Kyerematenng-Amoah et al., 2014). Although from some studies have investigated the pathogens noted above (*Camplyobacter jejuni, Chlamydophila psittaci, E.coli,* and Salmonella), relatively few published studies have assessed risk of other pathogen or antimicrobial resistant bacteria carriage among chicken slaughter / processing plant workers; even fewer have assessed this within US chicken slaughter / processing

plant workers. This lack of literature includes a lack of information about the gram-negative organisms in these environments, despite the recognition that these workers are heavily exposed to endotoxins, which originate from the outer cell wall of gram negative organisms. Systematic studies or reports of infections among these workers and workers in slaughter / processing plants of other poultry species are lacking; however, there are several reports of diseases and skin infections among these populations (R. C. Neyra et al., 2012; summarized in (Kyerematen-Amoah et al., 2014)). Several investigations of *C.psittaci* infections among workers in slaughter / processing plants have indicated that workers in contact with live birds or evisceration are more likely to become infected than workers with other duties within the plant (Deschuyffeleer et al., 2012). A health hazard evaluation, conducted at a Virginia poultry processing plant, observed that the majority 18/29 (62%) of their historical *Campylobacter* cases were live-hang employees, even though live-hang employees constituted only about 5% of the plant workers (de Perio, Niemeier, Levine, Gruszynski, & Gibbins, 2013). In combination with observed variations in airborne and carcass / product associated microbial counts, these two studies are suggestive that the risk to workers of infectious diseases may vary at different stages within the plant. A Dutch study compared resistance patterns of faecal enterococci among broilers, laying hens, farmers, and chicken slaughterhouse employees; it found that the prevalence of resistance correlated between the broilers and the slaughterhouse workers (van den Bogaard, Willems, London, Top, & Stobberingh, 2002). In Iceland, an analysis of *E.coli* isolates found the same antimicrobial resistance and pulsed-field gel electrophoresis patterns in bacteria isolated from broiler meat and a slaughterhouse worker at that plant (Thorsteinsdottir, Haraldsson, Fridriksdottir, Kristinsson, & Gunnarsson, 2010). In the Netherlands, a cross-sectional study found significantly higher MRSA prevalence in chicken slaughterhouse workers (5.6%) than in the general Dutch population (0.1%), with the highest MRSA prevalence found in workers with direct contact with the live animals (Mulders et al., 2010). These studies suggest that these workers are at risk of being exposed to bacterial pathogens from the broilers that are being

slaughtered and processed. Studies of pathogen or antibiotic resistant bacteria exposures in workers where other species are slaughtered further support this suggestion (Gilbert et al., 2012; R. C. Neyra et al., 2014; Van Cleef et al., 2010).

Nasal Gram Negative Organisms (GNOs)

Gram-negative organisms (GNOs) encompass a very diverse group of bacteria that inhabit a variety of niches in humans, animals, and the environment. This broad category includes commensal bacteria, opportunistic pathogens, and more virulent pathogens. Recently, certain species of GNOs have become of increasing public health concern due to high frequencies of multi-antibiotic resistance, particularly in health-care associated infections (Vasoo, Barreto, & Tosh, 2015). With respect to resistance, several GNOs are categorized as 'urgent threats' or 'serious threats' by the CDC (CDC, 2013a). These include resistant species that have mostly been observed in the settings of medical facilities, namely infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE), multi-drug resistant *Acinetobacter*, extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae,* and multi-drug resistant *Pseudomonas aeruginosa*, which are being tracked via CDC's National Healthcare Safety Network and Emerging Infections Program. Antimicrobial resistance in *Enterobacteriaceae* is of particular concern because of the ability of members of this family to easily share plasmids (Vasoo et al., 2015). Although these resistant organisms have mostly been studied in healthcare facilities, several studies have detected these carbapenemase producing (Guerra, Fischer, & Helmuth, 2014) and ESBL-producing (EFSA Panel on Biological Hazards (BIOHAZ), 2011; Ewers, Bethe, Semmler, Guenther, & Wieler, 2012; Liebana et al., 2013; Seiffert, Hilty, Perreten, & Endimiani, 2013) species from food-producing animals and their environments, leading to calls for more systematic, active surveillance for carbapenem-resistant GNOs in within settings related to industrial food animal production (Woodford, Wareham, Guerra, & Teale, 2014). Other GNOs on the CDC's priority list are resistant *Campylobacter* and *Salmonella,* which are food-associated infections; resistant

Salmonella Typhi, which is associated with travel to developing countries; resistant *Shigella*, which is more common in children, men who have sex with men, and individuals with poor hygiene; and gonorrhea, which is sexually transmitted (CDC, 2013a).

The moist microenvironment within the anterior nares (E. A. Grice et al., 2009) is a reservoir of opportunistic pathogens, like *S.aureus* (Wilson, 2005). This reservoir can be clinically significant; for example, with *S.aureus*, nasal colonization has been linked with increased risk of *S.aureus* infection (Verhoeven et al., 2014; Von Eiff, Becker, Machka, Stammer, & Peters, 2001). Nares are exposed to microbes in the inhaled air and in drainage from the nasal cavity and sinuses (Lemon et al., 2010), as well as through hand-to-nose transfers (Wos-Oxley et al., 2010). Compared to other skin sites, the bacterial membership and structure of the nares microbiome has been noted to be among the most temporally consistent (E. A. Grice & Segre, 2011). In healthy adults, it has been observed that certain components of the microbiological communities in the nares correlate with components of the microbiological communities at various skin sites; however, within individuals, the microbiological communities of the nares are distinct from those of the skin (Oh, Conlan, Polley, Segre, & Kong, 2012). In fact, the microbiota of the nares in one individual is thought to be more similar to the microbiota of the nares in another individual than it is to the microbiota of any other skin site from that same individual (E. A. Grice & Segre, 2011). The majority of flora in the nose is made up of gram-positive species (Carroll, Brooks, Butel, Morse, & Mietzner, 2013). Sequenced-based studies of nasal microbiomes in healthy adults in the USA and Germany have detected a variety of GNOs as a part of the nasal microbiome (Frank et al., 2010; E. A. Grice & Segre, 2011; Human Microbiome Project Consortium, 2012; Lemon et al., 2010; Oh et al., 2012; Wos-Oxley et al., 2010). However, sequence-based methods are generally more sensitive and detect a wider range of organisms than culture-based methods (Oh et al., 2012). In the relatively small number of published studies in which culture-based methods were used to sample the nares of healthy individuals, the microbiota of adult nares has been observed to be predominantly gram-positive

bacteria, with GNOs being much less frequently observed. In a study done by Danish investigators, a 'virtual absence' of GNOs was reported, and the only GNOs detected were *Haemophilus* spp. and *Moraxella nonliquefaciens* (Rasmussen, Kirkeby, Poulsen, Reinholdt, & Kilian, 2000). A study that took nasal septum swabs of 101 healthy Swedish police volunteers reported that gram-positive bacteria predominated their sample; of the 191 isolates only 14 were GNOs - three *M.catarrhalis* isolates, eight moraxelliform rods, and three *Enterobacteriaceae* (Hulterström, Sellin, & Berggren, 2012). Another study, which sampled non-deployed healthy US military service members, found nasal GNOs in 4% of the participants (Vento et al., 2013). Relatively speaking, more work has examined GNO carriage in the context of hospital and health care settings; however, much remains to be understood about the origin and transmission of GNOs even in these settings (Westwood et al., 2014).

Study Rationale

A growing body of evidence exists for the transmission of pathogens and antibiotic resistant commensal bacteria from animals to workers in industrial food animal production. As discussed above, antibiotic resistance in GNOs is of increasing public health concern and evidence exists for the presence of antibiotic resistant GNOs in the industrial food animal production environment. Further research is needed to characterize human risk of exposure to antibiotic resistant GNOs from these settings.

Broiler production is of particular interest due to ever increasing consumer demands for chicken products. From a microbiological perspective, the chicken slaughter / processing plant environment represents a unique environment where microbes from the gut and diverse body sites of very large numbers of broilers are brought together. In the chicken slaughter / processing plant environment, many of the workers are in close direct contact with large numbers of live broilers, fresh carcasses, or chicken meat throughout their shift. Additionally, due to cross-contamination throughout the plant, they are in indirect contact with the broiler-associated microbes that flourish in the plant environment. Generally, occupational risks of infection for chicken slaughter /

processing plant workers are not well-studied; however, the available literature suggests that workers at earlier parts of the production line are exposed to higher levels of chicken-associated bacteria than those working in later parts of the production line. Potential risks due to worker exposure to pathogens are especially concerning in light of high injury rates in this industry. Despite these potential concerns, very few studies have assessed occupational exposure to infectious diseases in US chicken slaughter / processing plant workers.

To the knowledge of the investigators, the cross-sectional exploratory study from which this data was obtained is the first epidemiological study in the US to assess nasal carriage of pathogens or antimicrobial resistant bacteria by poultry slaughter / processing plant workers. During processing of the nasal swabs from the subjects enrolled in the first day of study enrollment, high rates of overgrowth by GNOs during sample culturing for *S.aureus* was noted. Therefore, samples from participants enrolled on the following days (90 out of the 110 participants) were also assessed for GNOs (You et al., *unpublished manuscript*). Using the data obtained from these 90 participants, this analysis assesses whether there is a relationship between job duties and culture-detected nasal GNO carriage. It also explores the antimicrobial resistance patterns of the detected nasal GNOs. The results of this analysis contribute to the characterization of human risk of exposure to antibiotic resistant GNOs within the industrial food animal setting, particularly within the US chicken slaughter / processing plant environment.

Methods

Study setting: This analysis uses data from a cross-sectional study of unionized workers at the Columbia Farms broiler slaughter and processing plant in Columbia, South Carolina. This plant has approximately 775 employees; of those, approximately 635 (81.9%) are unionized and represented by the United Food and Commercial Workers International Union (UFCW). Per UFCW contacts, all workers in the plant at the time of recruitment were able to speak English; prior to the study, many of the Hispanic workers had left following recent immigration-related government inspections of the plant. Plant operations are organized into three shifts. During the first and third shifts, broilers are received, killed, and processed; only sanitation and cleaning activities occur during the second shift. Shifts vary slightly by department, with Quality Control shifts from 6:00am-3:00pm (1st shift) or 8:30pm-5:30am (3rd shift); Live Hanger shifts from 6:30am-3:00pm (1st shift) or 9:00pm-5:30am (3rd shift); Evisceration shifts from 7:00am-3:30pm (1st shift) or 9:30pm-6:30am (3rd shift); Deboning shifts from 8:45am-5:45pm (1st shift) or 12:00am-8:30am (3rd shift). The 2nd shift, or Sanitation shift, is either from 3pm-10pm or 4:30pm-11pm (Table M1). Per verbal descriptions of the plant layout provided by workers and an on-site observation conducted by a member of the research team, the processing areas of the plant are not located in separate rooms. Sections of the processing line are instead located in one large room, with partitions separating some sections.

Study design: This analysis used data from a cross-sectional study, and full details of the larger study are described elsewhere (You et al., *unpublished manuscript*). Local UFCW representatives informed Columbia Farms of the study prior to study initiation. Prior to the initiation of enrollment, the survey used for data collection was developed and pilot tested on six of the unionized workers. In partnership with the UFCW, which represents this workforce, workers were voluntarily enrolled to the study through UFCW health and safety officials at the national office and local UFCW representatives in South Carolina. Through notices and personal communication, plant workers from all shifts were informed of the scheduled enrollment times

and location by the local UFCW representatives and shop stewards. Due to the methods used to inform workers of the study, the total number of workers invited to participate is not recorded. Enrollment occurred at a local church within walking distance of the plant, and occurred over two recruitment rounds (3 days in Nov 2013 and 1 day in Apr 2014). All data obtained was collected via a survey and a nasal swab of each participant. The study was reviewed and approved by the Johns Hopkins School of Public Health Institutional Review Board.

Participant enrollment and study procedures: Workers eligible for study entry were currently employed at Columbia Farms, ≥18 years of age, able to understand an orally administered questionnaire in English, and willing to undergo a nasal swab. Participants were enrolled after verbally confirming their consent to a form that was read to them. In order to protect confidentiality and mitigate any concerns participants might have about honestly describing working conditions at the plant, personal identifiers such as name and exact date of birth were not collected. Neither the union nor Columbia Farms was not provided with access to the database or to individual-level survey responses; however, summary reports of selected portions of the data will be provided to the union. Collected survey data included participant demographics, occupational duties and work practices, recent contact with medical care, access to medical care, contact with animals or animal manure outside of the plant, community / household information related to risk of exposure to bacteria of nosocomial or agricultural origin, typical diet, household visitor information, and information related to household members' occupations and recent contact with medical care. Per the research team, most participants came to the enrollment site at the end of their work shift; however, the time of last shift was not measured as a part of data collection. Survey data was collected by trained interviewers, who administered the questionnaire to participants during one-on-one interviews and recorded participant responses on the paper questionnaires. Microbiological data was collected via nasal swabs taken from both nares of each participant. The 90 participants included in this analysis are those who were assessed for GNOs

(i.e. those enrolled after the first day); a subset of the GNOs isolated, as described below, underwent antimicrobial susceptibility testing (Fig. 1).

Microbiological analysis: Processing and microbiological analyses of the nasal swab samples are described in further details elsewhere (You et al., *unpublished manuscript*). Briefly, swab specimens were processed within 72 hours of collection. Swabs were processed and inoculated onto BBL™ CHROMagar™ Staph aureus agar (BD Diagnostic Systems) as well as BBL™ Trypticase™ Soy agar with 5% sheep blood (TSA II) (BD Diagnostic Systems) for incubation. *S.aureus* isolates were identified via latex agglutination (Pro-Lab Diagnostics, Ontario Canada); GNO isolates were identified via 16S rRNA gene amplification and sequencing, done after re-streaking to obtain pure colonies. One *S.aureus* isolate per positive individual and the GNO isolates with dominant morphologies (up to two per individual) were selected for antimicrobial susceptibility testing. Antimicrobial susceptibility testing was done on all selected *S.aureus* isolates and the GNO isolates belonging to the five most frequently detected genera (*Acinetobacter, Citrobacter, Enterobacter, Proteus, Pseudomonas)* in accordance with CLSI standards (CLSI, 2012). *S.aureus* isolates were tested for susceptibility to cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, virginiamycin, tetracycline, and trimethoprim-sulfamethoxazole. These antimicrobials were selected due to their clinical importance and their usage in poultry production (Silbergeld, Graham et al., 2008). GNO isolates were examined for susceptibility to a range of drugs, depending upon the species being tested: ampicillin, ceftazidime, ciprofloxacin, ertapenem, gentamicin, meropenem, piperacillin-tazobactam, tetracycline, and trimethoprim-sulfamethoxazole. These antimicrobials were selected due to their clinical relevance for the species. Isolates were classified as susceptible, intermediate, or resistant according to CLSI standards (CLSI, 2013). Isolates were defined as 'non-susceptible' if they met either the intermediate or resistant standard.

Data Entry and Management: A Microsoft Access database was designed for questionnaire data entry. Data from the paper questionnaires was manually entered into the database by two

members of the research team. Entered data was reviewed against the questionnaires by the assigned data entry personnel; furthermore, 20% of the data was verified via three rounds of double data entry, in which the double-entered data was entered by the other team member. Data entry errors and inconsistencies were discussed between the data entry personnel and rectified after each round of double data entry. Results from the microbiological analyses were manually entered into the Microsoft Access database by one team member and subsequently reviewed for data entry errors. Data was stored in the Microsoft Access database on the password protected laptop of the author (KL).

Analyzed Population: The population for this analysis consists of the ninety participants whose nasal swab samples were assessed for the presence of GNO. The twenty subjects whose nasal swabs were not assessed for GNO were not included in this sub-analysis.

Study Variables: *Job categories assignment (main exposure variable of interest).* Due to the heterogeneity of job descriptions within several job departments, and the large percentage of participants who reported working in an 'other' department, participants were categorized into job categories by the study team. Participants were initially assigned to five job categories: (1) contact with live animals, (2) processing, which includes evisceration, cutting, deboning, and sorting duties along the carcass processing line, as well as supervising and Quality Control of duties in these work areas; (3) maintenance / cleaning; (4) packing poultry products; and (5) other, namely shipping, box assembly, or office activities. The formation of these job categories was guided by the understanding that the processing activities did not occur in separate rooms, as noted during the formative research conducted by the study team; as well as by review of the self-reported department of employment and open-ended description of work duties in the questionnaire. These five job categories were defined based on assumed intensity of exposure to poultry, via direct contact as inferred from the questionnaire and knowledge about the plant layout, or via inhalation of bioaerosols as suggested by other studies (Liang et al., 2013; Lues et al., 2007; P. Whyte et al., 2001a). The order of the categories, by assumed intensity of exposures

(highest to lowest) was: 1) contact with live animals, 2) processing, 3) maintenance / cleaning, 4) packing, 5) other.

Two members of the study team (including author: KL) reviewed the open-ended job descriptions and departments from the questionnaires, and assigned participants into these job categories by consensus. Categorization was primarily guided by the descriptions; the reported departments were used as context. Individuals whose job descriptions entailed catching or hanging live chickens were categorized to the 'contact with live animals' category. Individuals whose job descriptions entailed evisceration, cutting, deboning, inspecting chicken body parts, recovering fallen parts and putting them back on the assembly line, supervising individuals conducting such activities, or conducting Quality Control of plant activities were categorized to the 'processing' category. Individuals whose job descriptions entailed machine maintenance, machine cleaning, floor cleaning (note: those whose job duties entailed recovering fallen parts and putting them back onto the belt were categorized as 'processing'), general cleaning, or cleaning supplies management were categorized to the 'cleaning and maintenance' category. Individuals whose job descriptions entailed packing, bagging, or weighing (note: per workers report, these activities include reaching into packing containers to adjust the amount of poultry product in the container) were categorized to the 'packing' category. Individuals whose job descriptions entailed office work, shipping work (shipping boxes of the finished poultry product), or box assembly were categorized to the 'other' category.

For several participants, the department and job descriptions reported on the questionnaire were indicative of multiple types of work. For these individuals, the job category was assigned based on the type of work that was classified as having the highest intensity of exposure to live chicken, chicken carcasses, or bioaerosols. For example, someone who reported job duties related to hanging live chickens, sanitation activities, and shipping activities would be assigned to the category with the assumed highest exposure, namely the 'contact with live

animals' category. Tabulations of job descriptions and department by assigned job category are provided in Table M2.

Due to the small sizes of some of these initial five categories, they were collapsed, based on assumed exposure similarity, into three categories for analysis, namely 'pre-slaughter / processing,' 'maintenance / cleaning,' and 'packing / other.' Prior to collapsing, similarity in terms of microbiological outcomes was also checked (Fig. M1). The 'contact with live animals' and the 'processing' categories were combined to form a 'pre-slaughter/processing' category. The 'packing' and 'other' categories were combined to form a 'packing/other' category. The 'maintenance and cleaning' category was not combined with another category due to uncertainty about the intensity of exposure of individuals in this group compared to the other groups.

Outcome. Participants were categorized as positive for nasal GNO, the primary outcome of interest for this analysis, if at least one GNO isolate was detected from his or her nasal swab sample. As only a subset of the GNO isolates underwent antimicrobial susceptibility testing and multiple GNO isolates were detected from some individuals, only isolates (and not individuals) were categorized based on susceptibility vs. non-susceptibility to antimicrobials.

Detected Nasal S.aureus: Participants were categorized as positive for *S.aureus* if *S.aureus* was detected from his or her nasal swab sample. Participants whose *S.aureus* isolate met the definition for non-susceptibility or MRSA were categorized, respectively, as positive for non-susceptible *S.aureus* or MRSA.

Covariates and other variables of interest. Variables measured through the questionnaire representing basic demographic data, potential sources of nasal GNO carriage (distinct from job duties), and potential protections against nasal GNO carriage, as well as nasal *S.aureus* status, were considered for analysis. Unless otherwise noted below, none of the defined variables had missing values or required imputation. Although typical diet, household visitors, household members' occupations, and household members' contact with medical care are recognized as potential sources of nasal GNO carriage, because no association of these factors with job duties

was expected and because of the higher potential for measurement error of these factors, they were not further considered for analysis.

Demographics: As year, but not date, of birth was collected, age was approximated by subtracting the year of birth from 2014. Due to the small number of non-African-American / black participants, race / ethnicity was categorized as African-American / black or other. Education level was dichotomized at completion of high school or GED.

Occupational: Analysis of nose-covering personal protective equipment was done with a binary variable based on whether participants reported using either a dust mask, surgical mask, or face shield either 'always' or 'everyday' while at work. Face protections described as 'other' in the questionnaire were manually reviewed to assess whether they would consistently cover the nose; none of the reported 'other' face protections met this criteria. Participants who reported not using any of these masks and participants who reported using these masks either 'sometimes' or only during the winter were categorized as not having 'consistent face mask usage.' Working shift was initially considered as reported by the participants – either first, second, or third shift – and no adjustment was done for the variation of the starting and ending times defining shifts in various departments of the plant. Due to the small size of the second shift category and the cleaning schedule of the plant, working shift was collapsed to a binary variable defined as either third shift or other (first or second) shift. Length of working day was analyzed as a binary variable, whose definition was based on whether the participant reported an average working day of at least eight hours or less than eight hours. The definition for 'same job duties over the course of a month' was based on whether the participant reported doing the same jobs or different jobs over the course of a typical month at work. For participants who reported having a second job outside of the plant, the open-ended job descriptions were reviewed. Recruitment rounds occurred during 14-16 Nov 2013 and 3 Apr 2014 (however, individuals recruited on 14 Nov 2013 were not assessed for GNO); for the purposes of analysis, recruitment rounds were dichotomized to Nov 2013 and Apr 2014.

Medical: Contact with health care was defined as answering yes to either of the following two situations within the past six months: "been admitted to a hospital or other medical facility as an inpatient for any reason (such as scheduled or emergency surgery or other treatment)" or "gone to a hospital or other medical facility, but not been admitted, such as for a doctor's appointment or to visit a family member or friend". Any reported antibiotic usage in the last six months, regardless of whether the participant also reported contact with health care, was defined as antibiotic usage. Reported MRSA diagnosis within the past year was assessed as a binary variable.

Household / community: Pet ownership was defined as a binary variable based on whether the participant reporting having any animals at his / her home or property. Animal butchering outside of work was analyzed with a binary variable based on whether the participant reported such behavior in the last six months. Participants were also grouped as 'living on a farm or nearby a farm or processing plant' based on whether they reported to living on a farm, near a farm, or near an animal processing or slaughter plant (near was defined as within 100 yards or 90 meters in any direction). One participant was unsure of living near a plant, but reported not living on/near a farm. This individual was categorized as not 'living on a farm or nearby a farm or processing plant'.

Statistical Analysis: The prevalence of nasal GNO (outcome variable) and nasal *S.aureus* by job category was determined for the ninety participants. The genera distribution of GNO isolates by job categories were examined. Among the *S.aureus* isolates and the GNO isolates that underwent antibiotic susceptibility testing, the proportion of non-susceptible isolates and patterns of antimicrobial resistance were assessed.

The distributions of variables representing demographic, occupational, medical, nasal S.aureus status, household-related, community-related, and study procedural information were examined and compared among the job categories (the five categories and the three collapsed

categories). Categorical variables were compared using Fisher's exact test and continuous variables were compared using one-way analysis of variance.

Unadjusted and adjusted logistic regression models were used to compare the odds of detecting any nasal GNO among the three collapsed job categories. Due to the relatively small sample size and the three job categories, the number of covariates included in the final model was limited to three.

To select covariates for the logistic model, variables representing potential sources of detected nasal GNO carriage or protections against detected nasal GNO carriage were considered. These covariates were age, gender, second jobs, consistent mask usage, length of working shift, homogeneity of job duties, shift, recruitment round (Nov 2013 or Apr 2014), contact with medical care in the last six months, antibiotic usage in the last six months, MRSA diagnosis in the last year, pet ownership, animal manure contact outside of work, animal butchering outside of the plant within the last six months, and residence on or near a farm or processing plant. Although recruitment round itself was not anticipated to impact actual nasal GNO carriage, this covariate was kept to include consideration of any unmeasured differences in study procedures or other factors that could have occurred between the two recruitment rounds.

Subsequently, the anticipated strength of the covariate's influence on the true association between job category and nasal GNO carriage, as well as the observed associations of the covariate with the three job categories and with nasal GNO carriage within this dataset were considered. Covariates for which a relatively limited influence was anticipated, and for which no or weak associations with the three job categories were observed in this dataset, were dropped from consideration. This resulted covariates representing the length of working shift and homogeneity of job duties being dropped from consideration. Next, covariates for which a stronger influence was anticipated, but which had no or weak association with the three job categories or nasal GNO carriage in this dataset were dropped from consideration. This resulted in dropping covariates related to second jobs, MRSA diagnosis within the past year, contact with

medical care in the last six months, animal manure contact outside of work, animal butchering outside of work within the last six months, and residence on or near a farm or processing plant. The unadjusted odds ratios of the remaining seven covariates (age, gender, consistent mask usage, shift, recruitment round, antibiotic usage in the last six months, and pet ownership) was then fit. Singly adjusted (job category plus one of the covariates) is included in Table M3.

Next, an over-fitted logistic regression model in which all of the remaining seven covariates and doubly adjusted (job category plus two of the covariates) logistic regression models were also fit and considered. Results from the over-fitted and singly adjusted models are in Table M4; graphical displays of the odds ratios for job category in the singly adjusted models are in Fig. S2. Selection of the covariates for the final model – gender, antibiotic usage, and shift – from these eight remaining covariates was based on consideration of: the association of the covariate with the three job categories, the strength of the adjusted associations of these covariates with nasal GNO carriage, and the impact of the adjusted association of job category with nasal GNO carriage in these models. The model's Pearsons residuals and predicted probabilities were used to graphically assess model fit; a sensitivity analysis in which the two observations with the largest DFFITS values were excluded was also examined.

To account for uncertainty about covariate selection, alternative logistic regression models which contained alternative sets of three covariates were fit and assessed. For these alternative logistic regression models, all ten possible combinations of the five covariates that either 1) impacted the odds ratio estimate for job category by more than 10% in the singly adjusted model or 2) contained a statistically significant odds ratio in the over-fitted model were checked. These five covariates were: age, gender, shift, recruitment round, and antibiotic usage.

Statistical and graphical analyses were performed using STATA version 13.1 (StataCorp, College Station, TX) and R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population: Of the 122 participants screened, ten refused participation due to time constraints, and two screen-failed due to their previous enrollment in the study. The ninety participants who were assessed from GNOs (Fig. 1) represented approximately 14.2% of the plant's unionized workforce at the time. They included workers who reported handling live broilers, carcass evisceration, cutting and processing fresh carcasses, cleanup and maintenance, packing, shipping, and office work. The majority of the participants were African American (86.7%) and male (64.4%) (Table 1).

Using the three collapsed job categories, the pre-slaughter / processing category had the highest proportion of African Americans (93.1%); the maintenance / cleaning category had the lowest proportion (66.7%) (p= 0.018, comparing all three groups; Table 1). The job category with the highest proportion of female participants was the pre-slaughter / processing category (46.6%) and the lowest proportion was in the maintenance / cleaning (13.3%) (p=0.013, comparing all three groups). Participants in the packing / other category were the youngest and those in the maintenance / cleaning category were the oldest (mean±SD: 37.0±8.6 vs. 49.1±12.8, comparing all three groups p=0.012). Although the percentage of participants reporting consistent face mask usage was similar across the three categories, mask usage appeared to differ by gender. Among the pre-slaughter / processing participants, 15/27 (55.6%) of the women, but only 7/31 (22.6%) of the men reported consistent face mask usage (p=0.015). In the other two job categories, statistically significant differences by gender in reported consistent face mask usage were not seen. The maintenance / cleaning category had the highest proportion of participants working before the third shift (86.67%); the packing / other category had lowest proportion of participants working before the third shift (17.65%) (comparing all three groups p<0.001). Pet ownership was most frequently reported among participants in the maintenance / cleaning category (53.3%) and least frequently reported among those in the pre-slaughter / processing category (22.4%) (comparing all three groups p=0.016). No statistically significant differences were observed

among job categories with regard to the other measured covariates. Nine participants in the cohort (10.0%) reported second jobs; however, the descriptions of these second jobs were not indicative of high risk of GNO exposure.

Prevalence of S. aureus, nonsusceptible S. aureus and MRSA

Among the ninety participants, the overall prevalence of nasal carriage of *S. aureus* was 15.6% (14/90) (Table 1). No significance differences in nasal *S.aureus* prevalence was observed between the three groups. The prevalence was similar in participants in the pre-slaughter / processing category (10/58 = 17.2%) and the packing / other category (3/17 = 17.7%), and lower in the maintenance / cleaning category (1/15 = 6.7%). Antimicrobial susceptibility was determined for these 14 *S. aureus* isolates. Six out of the 90 individuals carried *S. aureus* isolates that were nonsusceptible to any drug tested. Of those, one worker in the pre-slaughter / processing category, whose self-reported job description entailed removing poultry from the production line, carried an isolate that was classified phenotypically and genotypically as MRSA.

Prevalence of gram-negative organisms (GNOs) and nonsusceptible GNOs

Among the 90 participants, thirty-six were positive for nasal GNOs (40.0%) (Fig. 2a). By job category, 26/58 (44.8%) of participants in the pre-slaughter / processing category, 7/15 (46.7%) of participants in the maintenance / cleaning category, and 3/17 (17.6%) of participants in the packing / other category were positive for nasal GNOs (Fig. 2a).

Forty GNO isolates were obtained from these thirty-six participants, in most cases one isolate per individual. From four out of the 36 participants, two morphologically distinct GNO isolates were obtained. These four individuals were all in the pre-slaughter / processing category. *Acinetobacter* (11/40) was the most prevalent genus observed in the samples, followed by *Citrobacter* (7/40) and *Pseudomonas* (5/40). Less prevalent genera were: *Proteus* (4/40), *Enterobacter* (4/40), *Chryseobacterium* (3/40), *Klebsiella* (2/40), *Moraxella* (1/40), *Pantoea* (1/40), *Serratia* (1/40), and *Wautersiella* (1/40) (Fig. 2b and Table 2).

Antimicrobial resistance profiles for S. aureus and GNOs

In general, *S. aureus* isolates were susceptible to most of the tested antimicrobials. Six out of the 14 *S. aureus* isolates were non-susceptible to erythromycin; one of these was also resistant to cefoxitin (data not shown).

Antimicrobial susceptibility was conducted for 31 isolates from the five most commonly detected GNO genera, namely *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Proteus*, and *Pseudomonas* (Fig. 1 for study procedural flow; Fig. 3 for results). Two of these 31 isolates could not be regrown for testing. Of the 29 tested isolates, the overall proportion of nonsusceptible and resistant isolates was 9/29 (31.0%) and 8/29 (27.6%), respectively. Four out of the 29 isolates (13.8%) were nonsusceptible to two of the tested antimicrobials. The pattern of nonsusceptibility for the GNOs varied by genera (Fig. 3). The four *Proteus* spp. isolates (all *P. mirabillis*) were resistant to ceftazidime, and one of these was also resistant to ampicillin. Among the four growing *Pseudomonas* spp. isolates, three were resistant to trimethoprim-sulfamethoxazole. These three isolates were each also intermediate resistant to one other antimicrobial, either tetracycline or piperacillin-tazobactam. Among the ten growing *Acinetobacter* spp. isolates, one was resistant to trimethoprim-sulfamethoxazole and one had intermediate resistant to tetracycline. All *Enterobacter* spp. and *Citrobacter* spp. isolates were pan-susceptible.

Odds of nasal carriage of GNOs by job category

Using the final model, the adjusted odds of GNO carriage was 5.90 times (95% CI: 0.94, 37.50) and 6.29 times (95% CI: 1.43, 27.71) higher in participants from the maintenance / cleaning category and from the pre-slaughter / processing category than in participants from the category of packing / other (Table 3). These odds are adjusted for gender, working in the third shift as compared to earlier shifts, and self-reported use of any antimicrobial in the last 6 months. Female gender and usage of antimicrobials in the last 6 months appear to have a protective influence on the odds of nasal GNOs. The adjusted odds ratio for working prior to the third shift appeared was indicative of a protective influence; however, the confidence intervals for this estimate were very wide. Accounting for these covariates strengthened the association between

job category and nasal carriage of GNOs that was observed in the unadjusted model. A sensitivity analysis which excluded the two observations with the highest influence on the data, as measured by DFFITS, provided similar results to the above (data not shown).

Alternative models that adjusted for all possible combinations of age, gender, working in the third shift as compared to earlier shifts, and self-reported use of any antimicrobial in the last 6 months resulted in similar inferences; however, in two of the alternative models, the 95% confidence interval of the odds ratio for the pre-slaughter / processing category crossed the null (Fig. 4). In the model adjusted for age, working in the third shift as compared to earlier shifts, and self-reported use of any antimicrobial in the last 6 months, the adjusted odds ratio for participants in the pre-slaughter / processing category was 3.94 (95% CI: 0.96, 16.18). In the model adjusted for age, recruitment round, and self-reported use of any antimicrobial in the last 6 months, the adjusted odds ratio for participants in the pre-slaughter / processing category was 3.38 (95% CI: 0.83, 13.74). With the exception of the model that adjusted for age, recruitment round, and self-reported use of any antimicrobial in the last 6 months, the adjusted odds ratio for the pre-slaughter / processing category was larger than the unadjusted odds ratio. The adjusted odds ratio for the maintenance / cleaning category was more variable; in some models it was lower and in others it was higher than the unadjusted odds ratio.

As in the final model, in alternative adjusted models that contained gender, gender was consistently associated with a statistically significant (at the p=0.05 level) reduction in the odds of nasal GNO carriage (data not shown). In the final model and in the alternative models containing self-reported recent antimicrobial usage, recent antimicrobial usage was consistently associated with a protective effect; however, it was only of borderline significance ($0.05<p<0.10$) for the final model and one of the alternative models containing this covariate (Table 3 for final model results; data not shown for alternative models). In the final model and alternative models containing shift, working before the third shift was consistently associated with a protective effect. In the alternative models containing age, increasing age was consistently associated with

increased risk; however, there was considerable uncertainty (p>0.10) for this estimate in all the models containing age (data not shown). In the alternative models containing recruitment round, recruitment in April was consistently associated with reduced odds of nasal GNO carriage; in two of the six models containing this covariate, there was considerable uncertainty (p>0.10) for this estimate (data not shown).

Discussion

Relevance of Findings

This analysis examined cross-sectional associations between nasal GNO carriage, detected via non-selective culturing of nasal swabs, and inferred work-related intensity of exposure to broilers among workers at a broiler slaughter / processing plant. Exposure to broilers could have been related to either direct contact or to inhalation of bioaerosols. Due to the relatively small number of workers who were positive for nasal *S.aureus* (14 out of the 90 participants), a similar analysis was not feasible for *S.aureus* carriage.

Among the cohort as a whole, culture-detected nasal GNO carriage was common, with 36 (40%) of 90 participants positive for nasal GNOs. Relatively little is known about nasal GNO carriage in non-hospitalized individuals, and this appears to be the first study of GNO carriage among US poultry workers, so comparable results are not readily available from the literature. However, the frequency of nasal GNO carriage in this study population contrasts with the infrequency of nasal GNO carriage reported in the few other culture-based studies available in the literature (Hulterström et al., 2012; Rasmussen et al., 2000; Vento et al., 2013) and with textbook accounts of the nasal microbiome (Carroll et al., 2013) pg.167). As noted in the methods section, only the colonies with the dominant morphologies (up to two per individual) on the culture plates were used as isolates for further analysis. The presence and frequency of other morphologically distinct colonies on the plates was not recorded. Because of the limitations of this sampling methodology, it is not possible to draw conclusions as to whether the detected strain(s) of GNOs were the predominant bacterial species relative to other bacterial species in the nares at the time of the swab. The most recently available data for national prevalence of *S.aureus* in the United States is from the 2003-2004 National Health and Nutrition Examination Survey (Gorwitz et al., 2008); the prevalence observed in that survey was 28.6%, which is nearly double the prevalence observed in this study. Two participants in the pre-slaughter / processing category tested positive for both nasal *S.aureus* and GNOs. Besides these two participants, there appeared to be an

inverse relationship between carriage of *S.aureus* and GNO. This observation might reflect an artifact of competition either *in situ* or *in vitro* during culturing of isolates.

Many of the GNO genera detected are consistent with the results of studies in which either broiler carcasses, chicken meat, or air from slaughterhouses were sampled. *Enterobacteriaceae* species are present in high levels of the gastrointestinal tracts of broilers (Rehman et al., 2007); *Acinetobacter* and *Pseudomonas* species are among the primary bacterial isolates obtained from broiler carcasses in the processing line and in refrigeration storage conditions, respectively (Hinton et al., 2004); *Enterobacter, Citrobacter, Klebsiella, Serratia*, and *Pantoea* have been detected from chicken meat sampled at a German slaughterhouse (Schwaiger, Huther, Hölzel, Kämpf, & Bauer, 2012); and *Enterobacter, Klebsiella, Serratia, and Proteus* have been isolated from retail chicken meat in Europe (Kola et al., 2012; Overdevest et al., 2011). As noted in the background section, several studies in Asia and Europe have reported GNOs as airborne microorganisms in broiler slaughter and processing plants, including *Pseudomonas* (Liang et al., 2013; Lues et al., 2007); and *Acinetobacter, Enterobacter, Proteus, Moraxella, Klebsiella* (Ellerbroek, 1997); and general *Enterobacteriaceae* (species-level identification not done) (P. Whyte et al., 2001a). Some of these GNO genera have also been reported in air sampling done in chicken growing houses (Bakutis, Monstviliene, & Januskeviciene, 2004; Lawniczek-Walczyk et al., 2013).

Even though a reference group outside of the broiler processing plant was not available for this exploratory study, by showing an association between intensity of occupational exposure to broilers and nasal GNO carriage, these results provide preliminary support for the hypothesis that exposure to broilers within the slaughter / processing plant environment results in sufficiently high levels of GNO exposure to increase workers' risk of at least transient nasal GNO carriage. Adjusting for gender, self-reported antibiotic usage in the last 6 months, and working shift, the participants in the pre-slaughter / processing category, whose job duties entailed intensive exposure to broilers, had significantly increased odds of nasal GNO carriage (adjusted odds ratio:

6.29, 95% CI: 1.43, 27.71) compared to the reference group of participants working in packing, shipping, or office roles at the plant, whose job duties entailed less intensive exposure to broilers. Significantly increased odds for the pre-slaughter / processing workers were also seen in seven out of the nine alternative models used. The two models in which the increases were not significant did not adjust for gender, which appears to be a strong confounder of the relationship between job duties and nasal GNO carriage for workers in this category, perhaps due to differences in day-to-day work experiences between genders, and is further discussed below. Participants with maintenance or cleaning responsibilities, whose exposure to broilers is likely more variable, also appear to have higher odds of nasal GNO carriage than the participants in the packing / other category. However, the uncertainty in this estimate is greater and it was not statistically significant in the final model or most (eight out of nine) of the alternative models. At least part of this uncertainty is due to the relatively small sample size of this category (17 participants). Additionally, compared to the other categories, the demographics of this category were somewhat distinctive, as individuals in this category were more likely to be older, male, and a race / ethnicity other than African-American / black. One individual in this category, at the age of 76, was over ten years older than the second-oldest person in the cohort.

As noted in the introduction, GNO infections have become of increasing public health concern due to the detected rise in antimicrobial resistance in these organisms, particularly in healthcare associated settings but also in some community settings. Due to these concerns, risk factors for carriage of some antimicrobial resistant GNOs and the clinical implications of this carriage being further studied; however, this research is mostly for extensively resistant GNO carriage among individuals in frequent contact with health care (Vasoo et al., 2015). Among the isolates detected and tested in this study, nearly a third (9/29) displayed non-susceptibility to at least one of the antimicrobials tested.

Thirty of the detected GNO isolates were from *Acinetobacter* or *Enterobacteriaceae,* which are groups of bacteria in which antimicrobial resistance is of particularly high concern to

the CDC. None of the isolates displayed non-susceptibility to more than two of the antimicrobials tested, and none meet the antimicrobial pan-resistant definitions provided by CDC for 'important', 'urgent', or 'priority' threats (CDC, 2013a). Because the genera-level sample sizes are small, and it was not possible to conduct antimicrobial testing on all of the detected isolates, a more detailed analysis of the antimicrobial resistance patterns is not possible. Nevertheless, the substantial proportion of isolates displaying non-susceptibility suggests that GNOs with some antimicrobial resistance persist within the chicken slaughter / processing plant environment, and that workers – particularly those in frequent contact with broilers – are exposed to them in sufficiently high levels to establish at least short-term carriage. Given the high levels of concern surrounding antimicrobial resistance in GNOs in general, and in *Acinetobacter* and *Enterobacteriaceae* in particular, additional investigation of occupational exposures to these organisms and further characterization of their antimicrobial resistance patterns within the chicken slaughter and processing plant environment is warranted.

The high prevalence of nasal GNOs detected in this study appears to be unusual, but in contrast to nasal carriage of *S.aureus,* the clinical interpretation nasal GNO carriage is uncertain. The eleven genera of GNOs detected in this study include some species that are considered part of the normal microflora of other sites of the body and species that are known to cause opportunistic or nosocomial infections. Five of the *Enterobacteriaceae* genera detected – *Citrobacter, Enterobacter, Klebsiella, Proteus*, and *Serratia* – are part of the intestinal microbiota, and can sometimes be found in healthy upper respiratory or genital tracts in small numbers (Carroll et al., 2013, pg. 233), but species from these genera can cause a range of diseases (Carroll et al., 2013, pg.235; Vasoo et al., 2015). Species from the fifth *Enterobacteriaceae* genus detected, *Pantoea*, can be found in soil, plants, and feculent material, and certain species from this genera been reported to cause bacteremia, soft tissue infections, and bone / joint infections (Cruz, Cazacu, & Allen, 2007). The *Moraxellaceae* genera detected – *Acinetobacter* and *Moraxella* – can be present as commensals on the skin and mucus membranes

(*Acinetobacter*) or upper respiratory tract (*Moraxella*), but also can be pathogenic (Carroll et al., 2013, pg. 251). Species from *Pseudomonas* are widely distributed in nature, but certain ones can cause opportunistic infections (Carroll et al., 2013, pg. 245). The genera *Chryseobacterium* can occasionally colonize the respiratory tract (Carroll et al., 2013, pg. 251); relatively little information about the infection or colonization with the genera *Wautersiella* is available in the literature.

Thus, for all of the GNO genera detected in this study, carriage of species from these genera at other body sites is not a cause for concern in healthy individuals. Yet, most of these are known to be able to cause disease in susceptible hosts, and are not typically associated with the nares. The high levels of nasal GNO carriage among workers with intensive broiler contact suggests extensive exposures to GNOs within this environment. In conjunction with the high risk of injuries to workers in chicken slaughter / processing plants, this extensive exposure to opportunistic pathogens could be a cause for concern. Additionally, it is thought that the microbiota may protect against colonization by pathogens and the development of disease (Carroll et al., 2013, pg. 166; E. A. Grice & Segre, 2011); therefore, if these detected nasal GNOs do represent a disruption of a 'healthy' nasal microbiome, it could be speculated that this disruption might put the individuals at increased risk of disease.

Because of the small sample size, which precluded adjustment for more than three covariates in the model, and the lack of available literature on risk factors for nasal GNO carriage, multiple combinations of confounders were considered, as described in the methods section, to explore the sensitivity of the results to the assumptions made about confounding. Of the covariates explored, gender appears to be an important confounder of the relationship between categorized job duties and odds of nasal GNO carriage. Additionally, within the final adjusted model and the alternative adjusted models that contained gender, gender was consistently associated with a statistically significant (at the p=0.05 level) reduction in the odds of nasal GNO carriage. This may be partially explained by the observation that female participants more

frequently reported consistent usage of face masks in comparison to their male counterparts. There may also be additional unmeasured differences by gender in the actual work performed. Differences between men and women in issues related to day-to-day work experiences within the chicken slaughter and processing environment have been reported (Marin et al., 2009). As this study was conducted in a rural setting amongst Latino workers, it does not necessarily generalize to the setting of this broiler processing plant; however, it serves as an example of how day-to-day work performed might differ by gender. Beyond potential differences in how day-to-day work is performed by gender, microbiomes differ by gender (E. A. Grice & Segre, 2011); it might be speculated that this could lead to differences in susceptibility to nasal GNO carriage.

The adjusted effect of the other covariates in the final and alternative models was less certain than that of gender. The consistently protective effect of recent antibiotic usage against nasal GNO carriage in the final and alternative models containing this covariate is consistent with expectations. The observed protective effect of working before the third shift, which was consistently seen in the final and alternative models containing this covariate, was not anticipated as the plant is cleaned during the second shift, so the plant is presumably cleaner during the third shift than the first shift. It is possible that additional unmeasured differences between the day (first and second) and night (third) shift influenced nasal GNO carriage and resulted in this observed protective effect. However, there was considerable uncertainty about the direction of this estimate. Among the covariates not included in the final model, increased odds associated with increasing age and decreased odds associated with recruitment in April (as opposed to November) were consistently seen across all alternative models containing these variables, but both had considerable uncertainty in the direction of their estimates in at least some of the alternative models.

Methodological Considerations and Potential Future Directions

For this analysis, categorization of occupational broiler exposures was based on the self-reported department assignment and open-ended job descriptions, which varied considerably

across participants. Therefore, the day-to-day job duties represented by the collapsed job categories used in this analysis are relatively heterogeneous. Several participants reported doing multiple jobs, but did not provide descriptions of multiple jobs or indicate which job they were doing during their shift before study enrolment. Information about how frequently the individual did each job was also not recorded. It is possible that, had all participants who reported multiple jobs described the duties of their other job(s) or indicated which job they were doing prior to enrollment, some participants would have been categorized differently. For individuals within the maintenance / cleaning category, the reported responsibilities of individuals in this category ranged from responsibilities throughout all areas of the plant, such as managing and handing out sanitation supplies or machinery repairs, to responsibilities in a specific area of the plant, such as clearing debris and waste from floors or machinery within a given area.

The heterogeneity of job duties within job categories likely also corresponds to heterogeneity of exposure to broilers and broiler-associated bacteria. For example, within the maintenance / cleaning category, the individual whose job description was 'Clean the maestro - this is where the guts go after a machine takes them out,' likely has a much higher exposure to broiler carcasses and broiler-associated bacteria than the individual whose job description was 'passing out supplies' within the sanitation department. Within the packing / other category, individuals working in office or shipping roles would be expected to have much less exposure to broilers than those in packing. In considering the pre-slaughter / processing category, air sampling and carcass sampling studies done in other broiler slaughterhouses indicate that the airborne and carcass-associated bacterial counts vary considerably at different stages of the processing line, with generally speaking, higher counts during the receiving / killing and evisceration stages (Centers for Disease Control, 2014; Ellerbroek, 1997; Hinton et al., 2004; Liang et al., 2013; Lues et al., 2007; P. Whyte et al., 2001a). For *Campylobacter* and *C. psittaci*, outbreak investigations also provide some evidence of increased risk of infection to workers in these early parts of the processing line compared to the other workers (de Perio et al., 2013;

Deschuyffeleer et al., 2012). A study done in Dutch hog slaughterhouses also indicated individuals in contact with live animals were at higher risk of nasal MRSA than workers involved in later stages of slaughter / processing (Van Cleef et al., 2010). These studies suggest that, within the pre-slaughter / processing category, individuals working within the live hanging and evisceration areas of the plant might be expected to have higher exposure to broiler-associated bacteria than individuals working in later stages of the processing line. However, in many of the studies noted above, the descriptions provided in the publications suggest the plants were large with different rooms for the different stages of processing. This processing plant, on the other hand, was reported to have a relatively open layout, which would be expected to reduce the differences in airborne bacterial exposures between individuals in earlier vs. later stages of the processing line.

The limitations associated with one-sentence self-reported descriptions of job duties and the small sample size of this exploratory study precluded analysis of more granular categories of job duties. However, as each of the job categories analyzed, including the reference category, likely contains a mixture of individuals with relatively higher and lower levels of broiler exposure compared to others in the category, I would hypothesize that this heterogeneity within the job categories used for this analysis attenuates the estimate of the differences in odds of nasal GNO carriage between the least exposed and most exposed category. If a larger sample of the same population was available and more granular categories were used, more dramatic differences in odds of nasal GNO carriage between the least exposed and most exposed category might be expected. Additionally, the responses about job duties provided in this exploratory study could be used to design a questionnaire with more targeted questions about job duties for a larger follow-up study, so that the categorization based on job duties could be a better surrogate of the participants' actual exposure to broilers. These categorizations would be further improved if supplemented with air sampling results from plant work areas; however, it might not be feasible for researchers in the US to gain sufficient access to the plants to conduct air sampling.

In addition to the above limitations associated with categorizing exposure to broilers, because so little is known about nasal GNO carriage, there is a higher probability that confounding factors relevant to GNO carriage were not identified and measured during the data collection. For example, although the study team reported that most participants appeared to be coming to enrollment straight after work, it is possible that some workers came to enrollment prior to work or following a day off. The impact this unmeasured factor would have on the detection of nasal GNOs depends on how transient GNO carriage is after exposure. As the analysis is of a small observational dataset, and factors leading to nasal GNO carriage are not well understood, confounding may persist despite the statistical adjustments done during the analysis. A follow-up study with a larger sample size, including a reference population from outside the plant and more consistent sample sizes for each job category, would allow for the adjustment of additional confounders and would yield more conclusive results. Additionally, obtaining samples from multiple body sites, such as from the skin or from fecal samples, and sampling participants over time would provide a more complete picture of GNO exposure and carriage in the workers.

Participants in this study represent a convenience sample from the plant's unionized workforce, and therefore might not be representative of the larger population of plant employees. As a relatively small, urban processing plant with a largely unionized workforce, conditions inside this processing plant might not be representative of conditions in other broiler processing plants in the US, where there have been trends of plant relocations to rural areas and increasing proportions of foreign-born workers (Marin et al., 2009). Differences in plant management polices across companies and plants might also impact worker exposure to GNOs and antimicrobial susceptibility patterns. Additionally, broiler microbiota is variable, and the different feeding regimens and antibiotic usage policies of different companies can be expected to have a substantial influence on its composition (Rehman et al., 2007; Torok et al., 2011). Different

feeding regimens might not be expected to change the genera of GNOs detected in this study, but could impact their antimicrobial susceptibility patterns.

Conclusions

Despite its limitations, the findings from this analysis are provocative and suggest the need for additional research on human exposure to and carriage of GNOs, including antibiotic resistant GNOs, within the chicken slaughter and processing plant setting. The results of this analysis suggest that workers with increased exposure to broilers are at increased risk of nasal GNO carriage, which is consistent with the hypothesis that workers are exposed to GNOs via the broilers. Although the sample sizes by genera are extremely small, the antimicrobial susceptibility results are also consistent with the hypothesis that a non-negligible proportion of the GNOs that workers in chicken slaughter and processing plants are exposed to have some clinically relevant antimicrobial non-susceptibility. The results of this exploratory study provide a starting point for further investigation, particularly for the species of GNOs in which antimicrobial resistance is a high priority for the CDC. More broadly, these results add to concerns that poultry may be a non-nosocomial source of potentially pathogenic and drug resistant GNOs. Given the increased concerns regarding drug resistant GNOs, additional research regarding GNO carriage in the general population, as well as exposure to GNOs within the broiler slaughter / processing plant setting should be considered. These findings are consistent with the growing body of evidence that workers in industrial food production are exposed to high levels of a diverse range of bacteria, some of which display antimicrobial non-susceptibility. Additional surveillance of these settings may be warranted.

Tables and Figures

Methodological Tables and Figures

Table M1. Times of working shift within the slaughter / processing plant, by department.

Department	Shift	Time
Quality Control	1st	6:00am-3:00pm
	3rd	8:30pm-5:30am
Live Hanger	1st	6:30am-3:00pm
	3rd	9:00pm-5:30am
Evisceration	1st	7:00am-3:30pm
	3rd	9:30pm-6:30am
Deboning	1st	8:45am-5:45pm
	3rd	12:00am-8:30am
Sanitation	2nd	3:00pm-10pm
	2nd	4:30pm-11pm

Table M2. Summary of open-ended job descriptions and self-reported department by assigned job category (before and after collapsing) for the full study cohort (n=110, includes those who were not assessed for GNOs). An embedded Excel file is stored beneath the summary table.

Collapsed job category	Expanded job category	Self-reported department(s)	Summary of self-reported job description	Frequency
Pre-slaughter / processing (n=62)	Contact with live animals (n=7)	Live hanging (n=6)	Handles live animals	6
		Multiple (live hanging, shipping, sanitation) (n=1)	Handles live animals	1
	Processing (n=55)	Debone (n=18)	Checks body parts or carcasses	2
			Directly cuts or debones or vacuums body parts	14
			Organizes / places poultry parts on belt	1
			Supervisor	1
		Evisceration (n=17)	Checks body parts or carcasses	4
			Directly cuts or debones or vacuums body parts	9
			Lead / 'any position'	1
			Organizes / places poultry parts on belt	2
			Supervisor	1
		Multiple (debone & other) (n=2)	Directly cuts or debones or vacuums body parts	1
			Organizes / places poultry parts on belt	1
		Multiple (debone, shipping, maintenance) (n=1)	Directly cuts or debones or vacuums body parts	1
		Other (n=14)	Directly cuts or debones or vacuums body parts	9
			Lead / 'any position'	1
			Organizes / places poultry parts on belt	4
		QC (n=3)	QC of product or samples	3
Maintenance / Cleaning (n=21)	Maintenance / Cleaning (n=21)	Debone (n=2)	Picking up or cleaning poultry / debris / fluids from the floor - not putting them onto belt	2
		Evisceration (n=2)	Machine cleaning and / or repairs	1
			Picking up or cleaning poultry / debris / fluids	1

			from the floor - not putting them onto belt	
		Maintenance (n=4)	General cleaning	1
			Machine cleaning and / or repairs	3
		Other (n=3)	Picking up or cleaning poultry / debris / fluids from the floor - not putting them onto belt	2
			Dumps barrels of fluid waste	1
		Sanitation (n=10)	General cleaning	2
			Machine cleaning and / or repairs	5
			Picking up or cleaning poultry / debris / fluids from the floor - not putting them onto belt	1
			Supplies management	2
Packing / other (n=27)	Other (n=10)	Maintenance (n=1)	Office	1
		Multiple (shipping & other) (n=1)	Jack operator or truck loader	1
		Other (n=6)	Jack operator or truck loader	1
			Shipping materials	4
			Temperature control in shipping	1
		Shipping (n=2)	Jack operator or truck loader	1
			Shipping materials	1
	Packing (n=17)	Debone (n=1)	Boxing or bagging poultry products / adjusting weight of product in box	1
		Other (n=11)	Boxing or bagging poultry products / adjusting weight of product in box	11
		Shipping (n=5)	Boxing or bagging poultry products / adjusting weight of product in box	5

For an excel file of the line-by-line self-reported data and corresponding assigned category, double click on the icon: JobCategoriesComparedWithSelfRepo

Figure M1. Comparison of nasal GNO status by the five un-collapsed job categories.

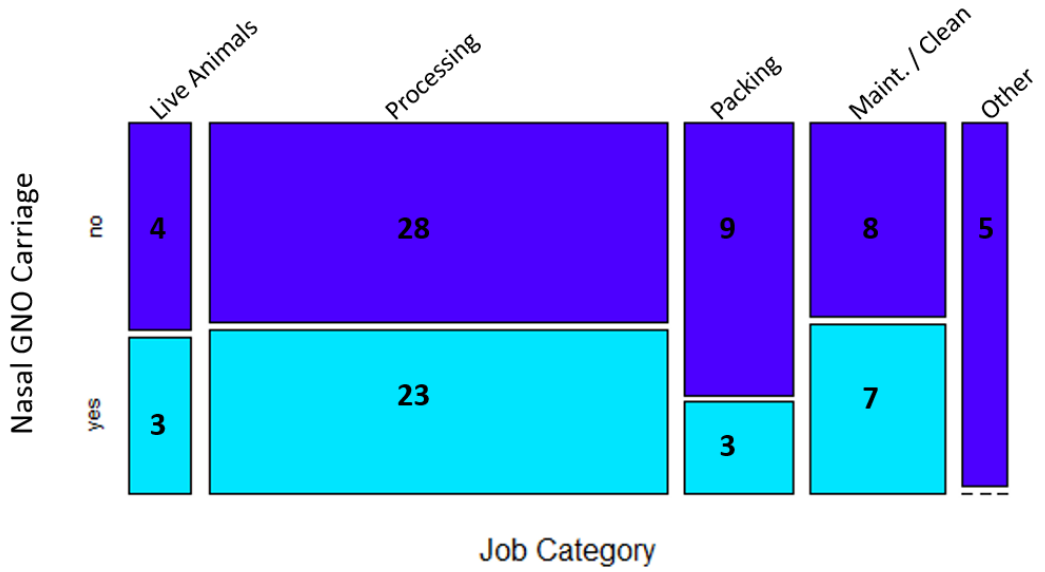


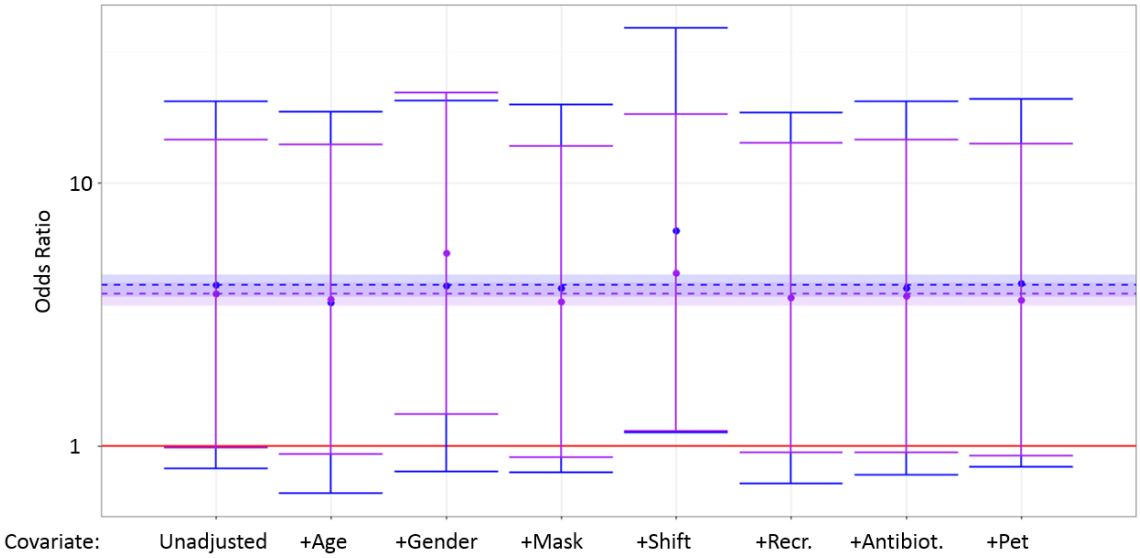
Table M3. Unadjusted odds ratios and 95% confidence intervals of final list of considered confounders with detection of nasal gram-negative organisms.

Covariate	n	Unadjusted odds ratio (95% CI)	p-value
Age (per 10 years)	90	1.21 (0.85, 1.74)	0.293
Female gender	32	0.45 (0.18, 1.14)	0.091
Consistent face mask usage	29	1.65 (0.68, 4.06)	0.271
Works before third shift	41	0.77 (0.33, 1.80)	0.546
Recruitment Month			
Nov 2013	30	Referent	---
Apr 2014	60	0.54 (0.22, 1.31)	0.173
Use of antibiotics in last 6 months	26	0.34 (0.12, 0.96)	0.041
Pet ownership	29	0.71 (0.28, 1.78)	0.462

Table M4. Adjusted odds ratios and 95% confidence intervals from over-fitted logistic regression model and logistic regression models containing one additional covariate.

		Adjusted Odds Ratio (95% CI)							
Category	n	Over-fitted Model	Model A	Model B	Model C	Model D	Model E	Model F	Model G
Job Category									
Packing / other	17	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Maintenance / cleaning	15	4.28 (0.57, 32.43)	3.51 (0.66, 18.60)	4.05 (0.80, 20.56)	3.97 (0.79, 19.90)	6.60 (1.12, 38.92)	3.65 (0.72, 18.56)	3.97 (0.77, 20.43)	4.16 (0.83, 20.88)
Pre/post slaughter animal contact	58	6.63 (1.32, 33.37)	3.61 (0.93, 14.04)	5.41 (1.32, 22.13)	3.52 (0.90, 13.77)	4.55 (1.14, 18.21)	3.66 (0.94, 14.22)	3.71 (0.94, 14.63)	3.59 (0.91, 14.08)
Age (per 10 years)	90	1.21 (0.79, 1.89)	1.14 (0.78, 1.66)	N/A	N/A	N/A	N/A	N/A	N/A
Female gender	32	0.17 (0.05, 0.61)	N/A	0.33 (0.12, 0.91)	N/A	N/A	N/A	N/A	N/A
Consistent face mask usage	29	1.46 (0.46, 4.58)	N/A	N/A	1.47 (0.59, 3.71)	N/A	N/A	N/A	N/A
Works before third shift	41	0.46 (0.15, 1.44)	N/A	N/A	N/A	0.51 (0.19, 1.36)	N/A	N/A	N/A
Recruitment Round									
Nov 2013	30	Referent	N/A	N/A	N/A	N/A	Referent	N/A	N/A
Apr 2014	60	0.24 (0.07, 0.98)	N/A	N/A	N/A	N/A	0.58 (0.23, 1.44)	N/A	N/A
Use of antibiotics in last 6 months	26	0.25 (0.07, 0.87)	N/A	N/A	N/A	N/A	N/A	0.35 (0.12, 1.00)	N/A
Pet ownership	29	0.70 (0.22, 2.25)	N/A	N/A	N/A	N/A	N/A		0.78 (0.29, 2.10)

Figure M2. Graphical display of the odds ratios and 95% confidence intervals for nasal GNO carriage, by the job category, in univariate and singly adjusted models. Purple corresponds to the estimates for the pre-slaughter / processing job category; blue corresponds to the estimates for the maintenance / cleaning category. The shaded areas show ±10% of the univariate odds ratio; horizontal lines at the null value (red) and the univariate values (dashed lines, color coded) are included in the graph for reference.



Main Tables and Figures

Fig. 1. Flowchart of study procedures. As detailed in the methods section, a subset of enrolled participants were not assessed for nasal gram-negative organisms (GNOs). Of the detected GNO isolates, 31 isolates belonging to the five most frequently detected genera underwent antimicrobial susceptibility testing. Note that in four participants, multiple morphologically distinct isolates (two in each participant) were identified.

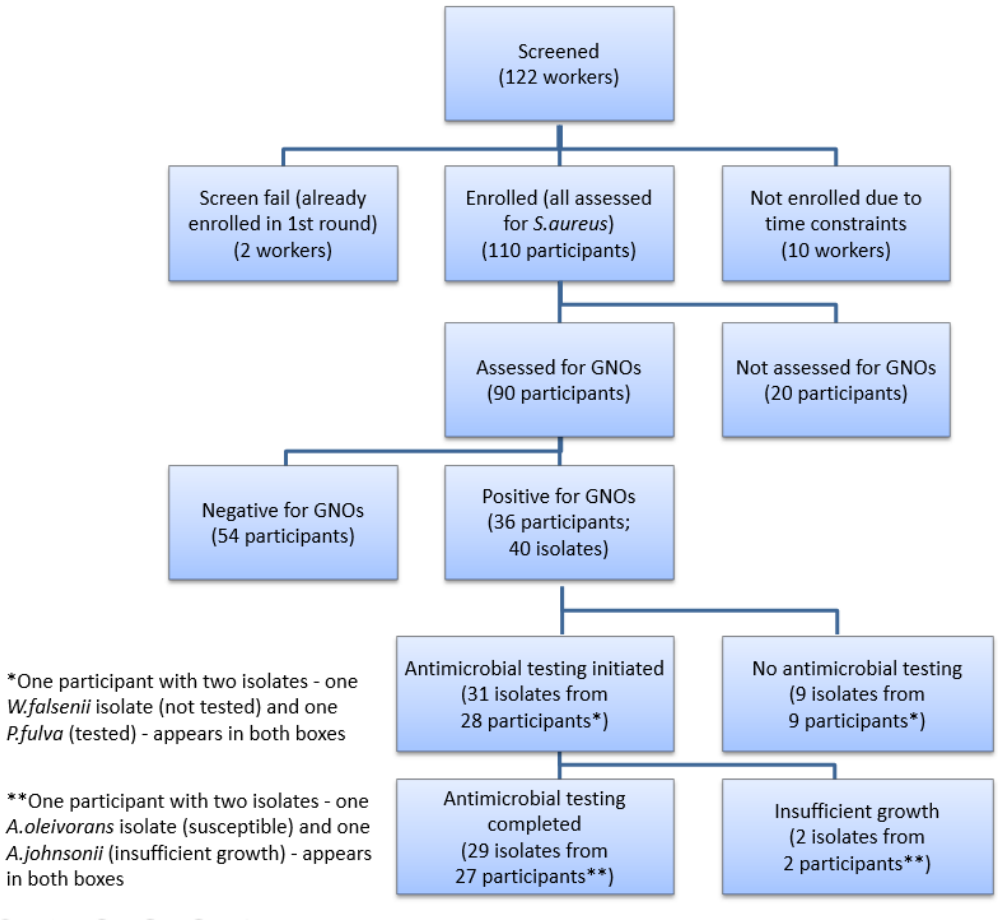


Table 1. Characteristics of analyzed population, by collapsed job categories.

Category	Total n=90	Pre-slaughter / processing n=58	Maintenance / Cleaning n=15	Packing / Other n=17	*p*-value[a]
Demographics					
Age – mean (SD)	41.6 (11.87)	41.0 (11.75)	49.1 (12.75)	37.0 (8.62)	0.012
Gender, female	32 (35.56%)	27 (46.55%)	2 (13.33%)	3 (17.65%)	0.013
Race/ethnicity					
African American	78 (86.67%)	54 (93.10%)	10 (66.67%)	14 (82.35%)	0.018
Other	12 (13.33%)	4 (6.90%)	5 (33.33%)	3 (17.65%)	
Education					
High school / GED or below	71 (78.89%)	46 (79.31%)	11 (73.33%)	14 (82.35%)	0.865
Beyond high school / GED	19 (21.11%)	12 (20.69%)	4 (26.67%)	3 (17.65%)	
Occupational					
Second job[b]	9 (10.00%)	7 (12.07%)	0	2 (11.76%)	0.513
Full time shifts (average working day ≥8 hours/day)	60 (66.67%)	37 (63.79%)	10 (66.67%)	13 (76.47%)	0.647
Same job duties throughout the month	69 (76.67%)	44 (75.86%)	12 (80.00%)	13 (76.47%)	>0.999
Works before 3rd shift[c]	41 (45.56%)	25 (43.10%)	13 (86.67%)	3 (17.65%)	<0.001
Season recruited					
Nov 2013	30 (33.33%)	19 (32.76%)	7 (46.67%)	4 (23.53%)	0.375
May 2014	60 (66.67%)	39 (67.24%)	8 (53.33%)	13 (76.47%)	
Self-reported consistent mask usage[d]	29 (32.22%)	22 (37.93%)	4 (26.67%)	3 (17.65%)	0.276
Males with consistent mask usage	*13/58 (22.41%)*	*7/31 (22.58%)*	*4/13 (30.77%)*	*2/14 (14.29%)*	
Females with consistent mask usage	*16/32 (50.00%)*	*15/27 (55.56%)*	*0/2*	*1/3 (33.33%)*	
p-value[a]	*0.010*	*0.015*	*>0.999*	*0.465*	
Medical					
Contact with medical care in last 6m[d]	58 (64.44%)	38 (65.52%)	8 (53.33%)	12 (70.59%)	0.592
Use of antibiotics in last 6m	26 (28.89%)	16 (27.59%)	4 (26.67%)	6 (35.29%)	0.792
MRSA diagnosis in the last year[e]	2 (2.22%)	1 (1.72%)	1 (6.67%)	0	0.341
Household / Community					
Pet owners	29 (32.22%)	13 (22.41%)	8 (53.33%)	8 (47.06%)	0.026
Animal manure contact (outside of work)	2 (2.22%)	1 (1.72%)	1 (6.67%)	0	0.341
Butchered an animal in the last 6m (outside of work)	4 (4.44%)	4 (6.90%)	0	0	0.614
Lives on or nearby a farm or processing plant[d]	8 (8.89%)	4 (6.90%)	1 (6.67%)	3 (17.65%)	0.367
***S. aureus* testing**					
S. aureus	14 (15.56%)	10 (17.24%)	1 (6.67%)	3 (17.65%)	0.695
Nonsusceptible *S. aureus*[f]	6 (6.67%)	5 (8.62%)	1 (6.67%)	0	0.701

[a]p-values calculated using Fisher's exact test for categorical variables and one-way analysis of variance test for continuous variables.

[b] Descriptions of reported second jobs are as follows: "tire business; custodial supervisor; volunteer cooperative ministry; restaurant; painting; construction; cleaning at University of SC; sale – used tires; [missing]"

[c] Live chickens arrive at the plant for the first and the third shift. During the second shift, plant cleaning occurs and no live chickens arrive

[d] See the SI text above for definition.

[e] Both diagnosed from a skin infection or wound.

[f] One of the non-susceptible isolates was a MRSA (with additional intermediate resistance to erythromycin); the other non-susceptible isolates were all non-susceptible to erythromycin.

Figure 2a. Nasal gram-negative organism (GNO) status, by job category and nasal *S. aureus* status, of the participants. Box width reflects number of participants in each job category while box height reflects percentage of GNO status. The number and percentage (including 95% exact binomial CI) of participants in each job category that tested positive for GNOs (of either *S. aureus* status) are included. In total, 36/90 (40%, 95% CI: 29.8%, 50.9%) participants were positive for nasal GNOs.

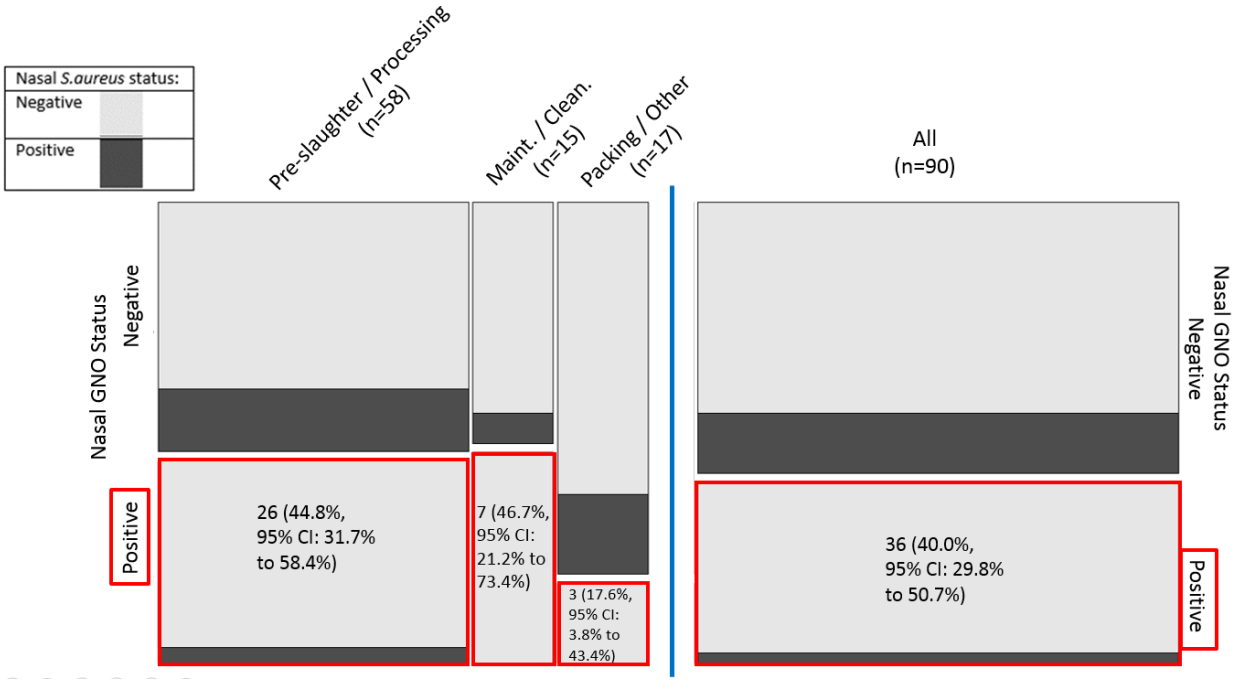


Figure 2b. Genera distribution of the 40 morphologically distinct GNO isolates and 14 *S. aureus* isolates detected from the participants, by job category. Box width reflects the percentage of isolates from each genus while box height reflects the percentage of isolates from each job category. Of these participants, 34 were positive for GNOs but not *S. aureus*, 2 were positive for both GNOs and *S. aureus*, and 12 were positive for *S. aureus* but not GNOs.

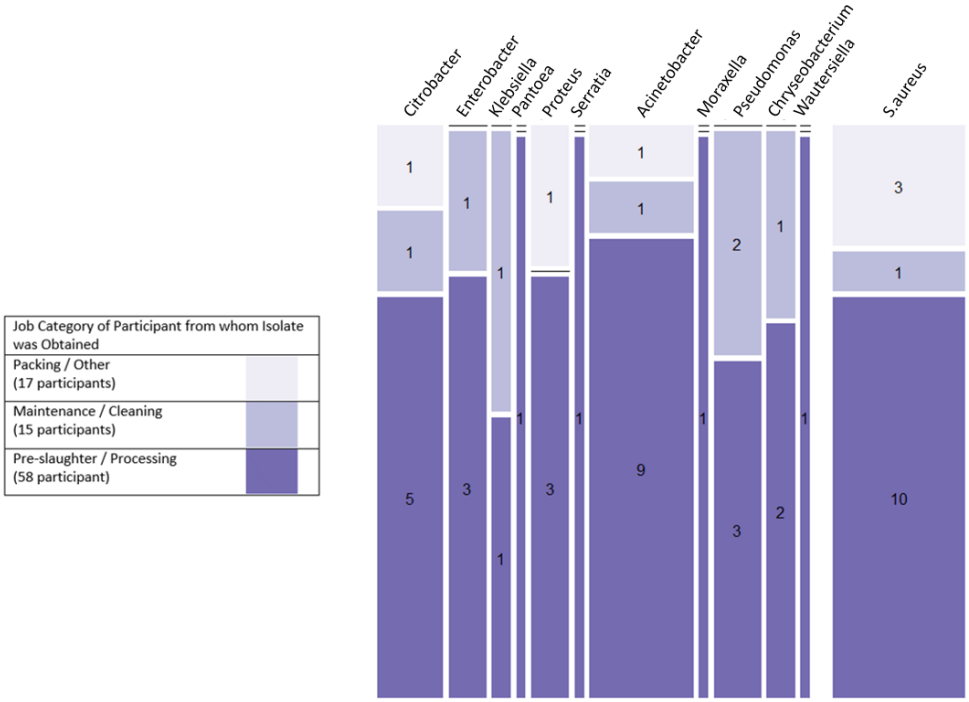


Table 2. Species-level listing of detected GNO isolates, by job category.

	Packing / other (17 participants)	Maintenance / cleaning (15 participants)	Pre-slaughter / processing (58 participants)	Species total
Citrobacter koseri	1	1	4	6 (15.0%)
Citrobacter freundii	0	0	1	1 (2.5%)
Enterobacter aerogenes	0	1	2	3 (7.5%)
Enterobacter sp.	0	0	1	1 (2.5%)
Klebsiella oxytoca	0	0	1	1 (2.5%)
Klebsiella pneumonia	0	1	0	1 (2.5%)
Pantoea sp.	0	0	1	1 (2.5%)
Proteus mirabilis	1	0	3	4 (10.0%)
Serratia marcescens	0	0	1	1 (2.5%)
Acinetobacter baumannii	0	0	2	2 (5.0%)
Acinetobacter calcoaceticus	0	0	1	1 (2.5%)
Acinetobacter johnsonii	0	0	1	1 (2.5%)
Acinetobacter lwoffii	0	0	1	1 (2.5%)
Acinetobacter oleivorans	0	0	1	1 (2.5%)
Acinetobacter radioresistens	0	1	1	2 (5.0%)
Acinetobacter sp.	1	0	2	3 (7.5%)
Moraxella sp.	0	0	1	1 (2.5%)
Pseudomonas fulva	0	0	2	2 (5.0%)
Pseudomonas putida	0	1	0	1 (2.5%)
Pseudomonas sp.	0	1	1	2 (5.0%)
Chryseobacterium culicis	0	1	0	1 (2.5%)
Chryseobacterium indologenes	0	0	1	1 (2.5%)
Chryseobacterium sp.	0	0	1	1 (2.5%)
Wautersiella falsenii	0	0	1	1 (2.5%)
Total isolates from job category	3	7	30	40 (100.0%)

Figure 3. Antimicrobial susceptibility pattern of the tested gram-negative organism (GNO) isolates. Forty GNO isolates were isolated from the nares of 36 out of the 90 participants, one per individual for 32 participants and 2 morphologically distinct isolates per individual for 4 participants. Of these isolates, we tested the antimicrobial susceptibility of isolates from the most frequently detected genera (*Acinetobacter* (n=10, 1 additional did not grow), *Citrobacter* (n=7), *Enterobacter* (n=4), *Proteus* (n=4), *Pseudomonas* (n=4, 1 additional did not grow). As indicated in the methods, the antimicrobials used in testing were based on the CLSI standard and consultation with the Johns Hopkins Hospital Clinical Microbiology Laboratory. Twenty out of the tested 29 isolates were pan-susceptible to all of the tested antimicrobials; 9 of the 29 isolates (31.0%) were non-susceptible to at least one antimicrobial. All *Enterobacter* and *Citrobacter* isolates were pan-susceptible to all of the tested antimicrobials and are not included in the figure below; the antimicrobial susceptibility patterns of the remaining 18 isolates are depicted in the figure. Some drugs were not tested for all genera due to intrinsic resistance or to not being recommended for those genera.

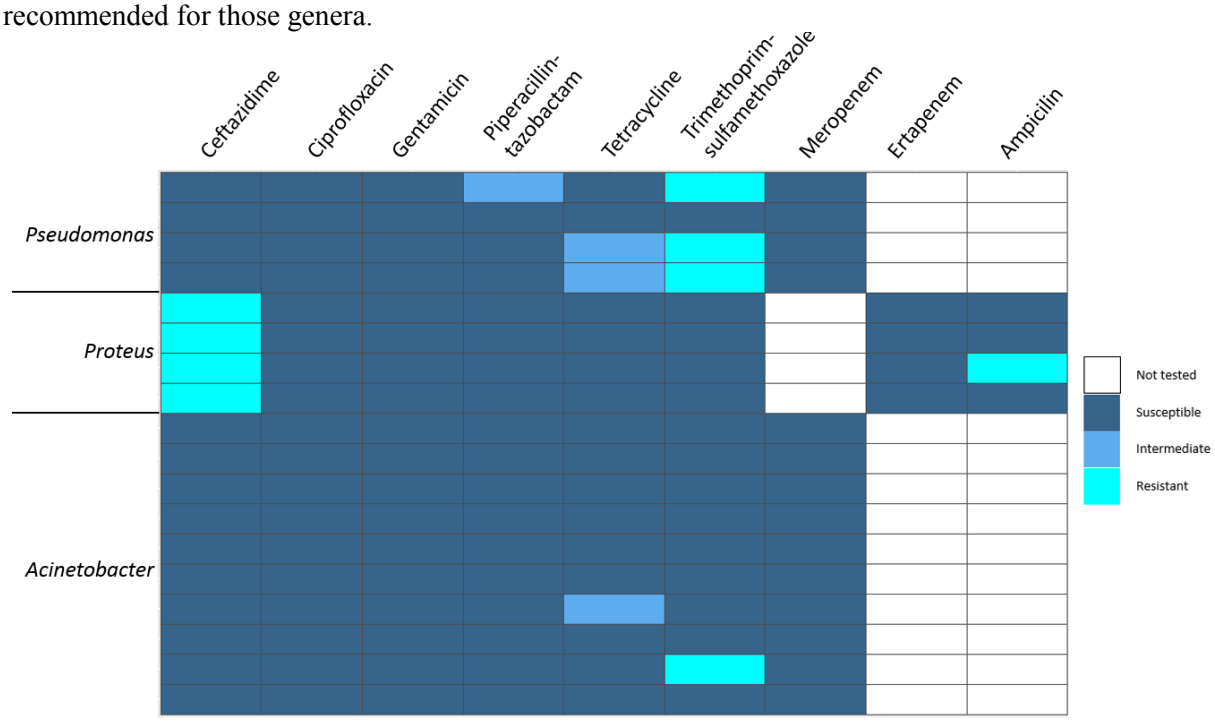
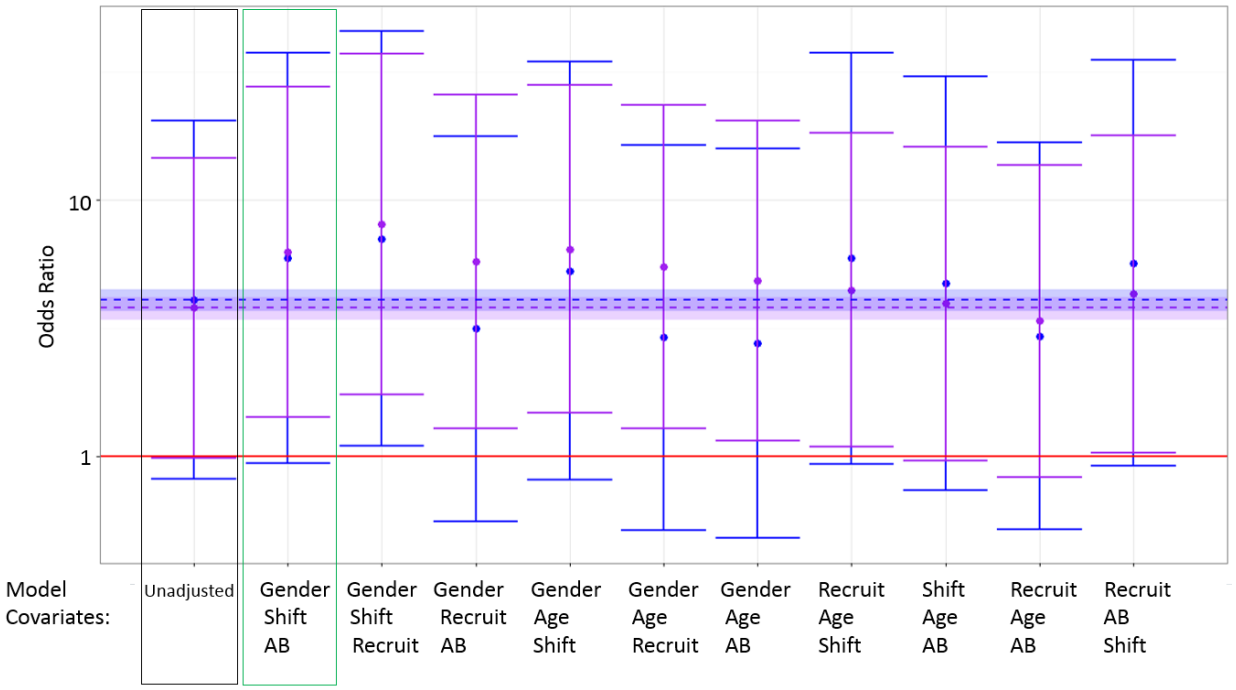


Table 3. Unadjusted and adjusted odds ratios, as well as the 95% confidence intervals, per the final selected model, estimating the association between work duties and covariates with detection of gram-negative organisms.

Category	n	Unadjusted odds ratio (95% CI)	*p*-value	Adjusted odds ratio (95% CI)	p-value
Job Category					
Packing / other	17	Referent	---	Referent	---
Maintenance / cleaning	15	4.08 (0.82, 20.38)	0.086	5.94 (0.94, 37.50)	0.058
Pre-slaughter/processing	58	3.79 (0.98, 14.63)	0.053	6.29 (1.43, 27.71)	0.015
Female gender	32	0.45 (0.18, 1.14)	0.091	0.33 (0.11, 0.93)	0.035
Works before third shift	41	0.77 (0.33, 1.80)	0.546	0.59 (0.20, 1.58)	0.272
Use of antibiotics in last 6 months	26	0.34 (0.12, 0.96)	0.041	0.39 (0.13, 1.15)	0.088

Figure 4. Graphical display of the odds /adjusted odds ratios and 95% confidence intervals for nasal GNO carriage, by the job categories, in univariate, final adjusted, and alternative adjusted models. Purple corresponds to the estimates for the pre-slaughter / processing job category; blue corresponds to the estimates for the maintenance / cleaning category. The shaded areas show ±10% of the univariate odds ratio; horizontal lines at the null value (red) and the univariate values (dashed lines, color coded) are included in the graph for reference. A black box and green box are added to the graph and enclose the unadjusted and final model, respectively.
Abbreviations: Recruit, recruitment round; AB, self-reported antibiotic usage in the last 6 months; shift, 3rd shift (shift after plant cleaning) or earlier shift.



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